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THE LIFE-HISTORY AND CYTOLOGY OF SYNCHYTRIUM ENDOBIOTICUM (SCHILB.), PERC., THE CAUSE OF WART DISEASE IN POTATO.

BY

K. M. CURTIS, M.A., M.Sc., D.I.C., F.L.S.

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IX.—*The Life-History and Cytology of* Synchytrium endobioticum (Schilb.), Perc., *the Cause of Wart Disease in Potato.*

By K. M. Curtis, *M.A., M.Sc., D.I.C., F.L.S.*

Communicated by Prof. V. H. Blackman, *F.R.S.*

(From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology.)

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INTRODUCTION.

Wart disease of the potato was first reported from Hungary in 1896 by SCHILBERSZKY (43), who gave a brief description of the summer and the resting sporangia, named the organism *Chrysophlyctis endobiotica*, and included it in the *Chytridineæ*. He saw the discharge of the zoospores from the summer-sporangia, and he put forward the view that the zoospores were responsible for the further distribution of the organism through the tumour by the ability which he believed they possessed of boring their way through the walls of the host cell into the adjoining cells. The fungus probably existed in England many years before SCHILBERSZKY'S paper was written, but its presence in this country was not generally recognised. In 1902 POTTER (36) published a short paper on the organism. He there showed that resting sporangia, which had been kept dry during the winter, were able to cause the infection of tubers the next season. The distribution of the organism through the tumour he attributed to its division, when in a condition which he describes as plasmodial, and to the passage of the segments so produced through the walls into the adjacent cells.

For several years after POTTER'S publication appeared few facts were added to the existing knowledge of the disease, but in 1907 BORTHWICK (4) reported that leaves could be attacked as well as tubers. In the following year, SALMON (42) carried out a series of infection experiments, from the results of which he concluded that resting sporangia, after exposure for $1\frac{3}{4}$ hours to a temperature ranging from $-5°$ C. to $-6°$ C., could dispense with the winter dormancy and germinate at once. Shortly afterwards two notes appeared, one from the pen of JOHNSON (16) and the other from that of WEISS (49). Both succeeded in obtaining the germination of the resting sporangium, and, in addition, JOHNSON found that the zoospores liberated exhibited the usual characteristics of the Chytridian zoospore, while WEISS remarked upon the

rapidity with which they became amœboid. In December of the same year MASSEE (31), when exhibiting specimens of diseased tubers at a meeting of the Linnean Society, expressed the opinion that the organism belonged to the genus *Synchytrium*; but he based his statement upon the so-called epidermal nature of the parasite and upon the supposed presence of an enveloping membrane round the protruding contents of the germinating resting sporangium, both of which suppositions have since been shown to be incorrect.

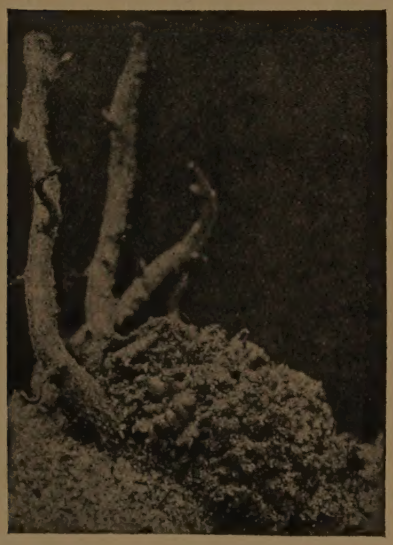
In the next year JOHNSON (17) questioned the validity of MASSEE'S statements, maintaining, on the one hand, that the organism may be deeply seated in the tissue of the tumour, and, on the other, that in the germination of the resting sporangium "the wall ruptures and the zoospores escape without any sign of the extrusion of a membrane." Not long afterwards appeared a paper by PERCIVAL (35), in which was made the first real contribution to our knowledge of the cytology of the organism. In the young resting sporangium the marked increase in size of the nucleus and nucleolus during the period of active growth, and the presence in the nucleus of an irregular structure called by PERCIVAL an "amœboid body," are briefly but plainly described. He believed that chromatin is transferred from the nucleolus to this amœboid body, and that about the time of the transference chromatin granules appear in the mesh of the cytoplasm, which then gradually breaks up into swarm-spores. These, PERCIVAL stated, were not always of the same size, and the possibility of an anisogamous fusion was suggested, although no union of any kind was observed. The method by which the secondary nuclei arose in the young sorus was believed to resemble that reported for the resting sporangium; the chromatin becomes associated with the linin in the nuclear vacuole, while the nucleolus loses its staining power and disappears. Chromatin meanwhile appears in the cytoplasm in the form of chromidia, and on the formation of vacuoles round these granules secondary nuclei are instituted. These small nuclei were found to undergo mitosis, and their behaviour was thus at variance with that of the primary nucleus. When the young sorus divides into its constituent sporangia the cleavage was believed to proceed from the periphery inwards. At the conclusion of the paper, on the basis of the morphological and cytological resemblance to members of the genus *Synchytrium* exhibited by the organism, PERCIVAL proposed that it should be placed in that genus under the name *Synchytrium endobioticum*.

The next noteworthy contribution to the life-history of the organism was published in 1912 by BALLY (1), who supported JOHNSON in his belief in the possibility of root infection of the host plant. In the resting sporangium this author ascribes the transference of chromatin from the nucleus to the cytoplasm to the migration into the cytoplasm of the granules which he observed studding the inner surface of the nuclear membrane. In the zoospores BALLY failed to find any trace of the change from the free-swimming to the amœboid condition such as had been reported by the earlier writers. With regard to the cytology of sorus, both macro- and micro-nuclei

were figured in the drawings, and mitosis of the primary nucleus, though not observed, was believed to take place. While BALLY preferred to retain the organism in the genus *Chrysophlyctis*, G. TOBLER (48), on the other hand, in a monograph of the genus *Synchytrium*, accepts PERCIVAL'S suggestion. For reasons to be given later, the organism in the present paper is also referred to the genus *Synchytrium*.

OUTLINE OF THE LIFE-HISTORY.

The fungus has no mycelium and at the reproductive period the whole body forms a sorus or a resting sporangium. In the spring the zoospores of the resting sporangium are liberated, and after a short period of activity they infect young tissue of the potato plant. The zoospore on entry passes to the lower end of the host cell, and there, after enlarging, develops a firm yellow outer, and a thin colourless inner, membrane (fig. 13). The protoplasm of the organism, while still in the uninucleate condition and enclosed in an expanding portion of the inner membrane, now passes through a small pore in the outer membrane into the upper portion of the host cell (figs. 30, 35). Repeated mitosis immediately takes place, and after five or six nuclear divisions the protoplasm segments into about five thin-walled sporangia, which together constitute the sorus (fig. 60). Further nuclear divisions occur and ultimately zoospores are formed. General division of the neighbouring cells of the host plant has meanwhile resulted in the formation of a tumour, while, in addition, the ring of epidermal cells in contact with the infected cell has grown up to form a rosette, whose projecting cells arch over the host cell lying at their base (figs. 2, 7). The sporangia when mature absorb water and enlarge rapidly, rupturing the soral envelope and the host cell wall, and so lie exposed to the exterior.



TEXT-FIG. 1.—Tumour on "Arran Chief." ($\times \frac{1}{4}$.)

The motile cells formed in the sorus have previously always been termed zoospores, but they may behave as gametes and fuse in pairs. The zygote resulting from the gamete fusion enters an epidermal cell of the potato plant, and there develops into a resting sporangium. By the division of the host cell, the resting sporangium becomes deeply placed in the tumour. Eventually the host cell dies, and its contents are deposited as a brown epispore upon the parasite (fig. 120). If fusion has been omitted, the facultative gametes ("zoospores"), on entering the plant, give rise to a sorus, and the zoospores developed in it either may or may not fuse. There is evidence that the treatment to which the maturing soral sporangia are subjected may decide whether zoospores or gametes are developed. In this paper the term

zoospore, for the sake of convenience, is retained as a general term for the motile cells formed in both soral and resting sporangia.

As it is impossible to determine the stage in the development of the sorus at which the organism passes into the reproductive phase, the term *prosorus* is applied to all stages between the completion of the rounding off of the zoospore (which takes place immediately after its entry) and the beginning of segmentation into sporangia.

Method of Investigation.

The most suitable varieties of potato for the purposes of inoculation were found to be Arran Chief, Mixed Early, and Midlothian Early. A reserve supply of diseased material was maintained by growing healthy tubers in soil from an infected field. Experimental work was conducted in the greenhouse, where the maintenance of a constant high temperature induced prolific growth in the tumour. It was essential that the development of the latter should be followed from day to day, and, although when tubers are planted sufficiently high in the soil, at least one malformation in each pot may be expected to reach the surface, where its subsequent growth can be watched, it was found more convenient to avoid the use of soil when the observation of a particular stage was desired. The most useful substitute for soil proved to be *Sphagnum*. The tubers were set to sprout under a light covering of the damp moss, but, at the first sign of growth in the eyes, sufficient *Sphagnum* was removed to leave the upper surface of the potato exposed. A drop of water containing active zoospores from the resting sporangium was then placed on one or two eyes in each potato, and for two days the plants were kept in a dull light and a saturated atmosphere. After this time they could be safely exposed to the normal conditions of the greenhouse. A few days after inoculation, small tumours were to be detected with the aid of a lens. The plants were then lightly watered once a day. At this stage the tumours bore only a few scattered sori, and, when stages in their development were required, the first, second, or even the third series of sori was permitted to discharge before portions of the tumour were removed for fixation. By that time the hypertrophy had attained a considerable size, and bore a plentiful supply of sori of all ages.

To induce the fusion of the soral zoospores, and so secure the formation of resting sporangia, the plants were not watered more often than once in four days. The object was to keep the soral sporangia undischarged and without water for at least two days after they had matured. On the addition of water, most of the zoospores, on being discharged, would fuse, and the greater portion of the new series would thus consist of resting sporangia. In the tumour, whose photograph is reproduced in fig. 1, the order of formation of reproductive bodies after control commenced was sori, sori, sori and resting sporangia mixed, sori, sori, resting sporangia. The resting sporangia do not germinate immediately, so, in the third generation of the above series, the zoospores of the sori were alone responsible for the production of the next

generation. Usually, not more than two series of sori and one of resting sporangia are formed in any particular region of a tumour, and in cases of natural infection in the soil large areas of the tumour may frequently be found, which seem to bear resting sporangia only. But, on careful examination, traces of a few ruptured sori can usually be found somewhere in the vicinity.

To secure stages of the entry of either form of zoospore, an abundant supply of slender shoots, less than $\frac{1}{4}$ inch long, is required. For this purpose clean tubers were planted in sawdust just beneath the level of the surface. When the shoots appeared above the surface, the plants were removed to a cool room, and water was withheld in order that growth might cease. Two days before the inoculation was to be made, however, they were thoroughly watered and replaced in the greenhouse. By the time they were required, each had produced great numbers of actively growing shoots of the desired size. A drop of water containing the zoospores was placed upon the apex of the shoot, and the plants were then kept in a saturated atmosphere until the time of fixation.

The fixative used was FLEMMING'S strong solution, diluted with an equal quantity of water. The reaction of the organism to stains varies widely, according to the stage of development. HEIDENHAIN'S Iron-alum-hæmatoxylin, FLEMMING'S Triple Stain, and BREINL'S Triple Stain, are all excellent for the staining of any particular stage. But only FLEMMING'S Triple Stain (or its simpler form, Gentian violet and orange G) can be relied upon to give uniformly good results when the examination of all the stages occurring on a slide is contemplated.

MORPHOLOGY OF THE PROSORUS AND SORUS.

The colour of a tumour growing above ground varies from white to deep green, according to the intensity of the illumination to which it has been exposed, and, if the potato belongs to a variety which normally has purple markings, the edges of the surface irregularities will probably be correspondingly coloured. By means of a lens, the presence of maturing sori can readily be detected, owing to the paler shade and minute granulation of the surface which bears them. Both of these characteristics are due to the presence of the projecting and highly refractive rosette cells surrounding each sorus.

In a surface view of the epidermis of the tumour under a low power objective, the young prosorus is to be distinguished as a densely granular sphere, with a thin, colourless wall, and a lighter area in the centre, where the nucleus lies. The host cell is much enlarged below, and little protoplasm can be detected in it. When the organism is quite young, the cells of the tumour adjoining the host cell do not project above the level of the surface, as may be seen in the three parasites to the left of the empty rosette in fig. 8. The wall of the prosorus gradually thickens, and becomes light golden-brown in colour. The same colour may soon be observed in the wall of the host-cell also, but in this case the change is probably due solely to the

deposition of the contents of the host cell upon it. Usually, only one prosorus occurs in each host cell, but two are not uncommon (fig. 29), and, on one occasion, four host cells, almost adjoining one another, were found to contain two, three, three, and four prosori respectively. Occasionally, prosori and resting sporangia lie side by side in the one cell.

The migration of the contents of the prosorus into the upper portion of the host cell, which does not take place until after the membrane has assumed its golden colour, may be slightly oblique (fig. 30), but the general direction is always upwards, the vacated membrane being invariably left below. The discharge is completed in about four hours and in favourable material the process can be watched under the microscope. The rate of movement of the protoplasm is at first very slow, but it increases rapidly as the process continues. (For stages of the migration see figs. 29–35.) The epidermal cells adjoining the host cell begin to enlarge about the time of the migration of the prosoral contents, and thenceforth they keep pace with the expansion of the host cell (fig. 12). They are refractive, turgescent cells and when viewed from above are seen to be arranged in a radial fashion round the depressed host cell at their centre (figs. 3, 4, 5). As they enlarge still further their free ends curve slightly inwards, arching over the host cell and forming a natural trap for the retention of moisture upon its surface. At maturity they are divided transversely by walls formed parallel to the surface of the tumour, and the width of the whole rosette is increased through the epidermal cells lying at the periphery undergoing a slight and relatively late enlargement (fig. 7). Cleavage of the prosorus takes place soon after its migration, and as the maturing sporangia absorb water and swell the host cell is subjected to great pressure. The cells of the host tissue immediately beneath it have meanwhile undergone repeated division in the direction parallel to the surface of the tumour, and as they also absorb water and swell at the same time as the sporangia, the upper wall of the already distended host cell bursts (figs. 67, 71). As the result of the rupture the upper sporangia are usually jerked out, but the others remain in the host cell and there effect the discharge of their zoospores (fig. 72*a*). The lower sporangia are the latest to mature and their zoospores may be liberated as late as thirty-six hours after the sporangia have become free. Those of the upper sporangia, on the other hand, are usually discharged immediately after the rupture of the host cell. After the rupture the crumpled remains of the outer wall are left as an irregular brown ring round the top of the cell (fig. 8).

The Prosorus.

The Cytology of the Prosorus.

As soon as the entering parasite lies wholly within the epidermal cell it assumes the spherical form which is regarded in this paper as the beginning of the prosoral stage. It then begins to pass towards the lower end of the cell (fig. 9). The means by which the parasite moves is somewhat obscure. There is certainly no indication of the

assumption of an amœboid form, the existence of which has been claimed by some previous writers. A perfectly spherical form is preserved throughout the period of movement and probably the organism is carried passively by the protoplasm of the host cell. The body of the prosorus at this stage consists of a few delicate strands of protoplasm radiating from the nucleus to the periphery and separated by large vacuoles. At the time when the downward movement in the cell begins the nucleus may not yet have regained its spherical form after the compression to which it was subjected in its passage through the wall (fig. 10*a*). It is now in a transitional stage between the condition of the nucleus of the zoospore, in which there is a clear central vacuole and the chromatin is confined to peripheral granules (fig. 74*b*), and that of the more mature prosorus, where the centre is occupied by a single large nucleolus and peripheral granules are absent or inconspicuous (fig. 19). The exact nature of this transitional stage is somewhat obscure. At this stage it is difficult to detect any structural differentiation in the nucleus. The outline is a little irregular and there are indications at its periphery of the original granules of the nucleus of the zoospores (fig. 10*b*). Staining material, however, is being formed so rapidly that soon all that can be observed is a dark homogeneous granule of spherical form (fig. 10*c*). A small clear area next appears round the granule (fig. 10*d*) and there is thus formed the normal vacuolated nucleus which persists until the first nuclear division occurs. Similar stages of nuclear development are to be followed more clearly in the parasite developing from the zygote (p. 445).

When the prosorus is established in its final position it commences to enlarge at an extraordinary rate and the nucleus, which increases in size even more rapidly, soon forms a strikingly large object in the centre of the organism. During the enlargement the amount of protoplasm has increased to a marked extent and the earlier arrangement in radiating strands is gradually replaced by a reticulum of uniformly small mesh (compare figs. 9 and 10).

The parasite has usually reached the lower end of the host cell before it causes any change in the size of the cell. Zoospores have regularly been found to congregate particularly on the surface of the tissue of which the epidermal cells are undergoing rapid division; in any group of such actively dividing cells those whose nuclei are actually engaged in the process of mitosis seem to be especially liable to infection. The effect of infection upon these host cells becomes evident earlier and usually is more marked than upon those whose nuclei are in the resting condition at the time of infection. The first effect produced by the presence of the parasite consists of a rapid enlargement, chiefly in the downward direction, so that the host cell becomes a large pyriform sac with its outer wall remaining for some time unaltered in size (fig. 11). By this time the protoplasm is reduced to a thin layer covering the parasite and the walls of the cell. The host nucleus, however, increases considerably in size, but except when compressed by the expanding prosorus it suffers no distortion until just before its death, which occurs somewhat later.

The structure of the nucleus of the prosorus now becomes more complicated as the result of a series of discharges of the substance of the nucleolus into the vacuole of the nucleus. The chromatin thus discharged gradually dissolves in the vacuole leaving behind a linin matrix. Before entering upon a more detailed description of the nucleolar discharges, however, the general appearance of the prosorus and host cell during that process will be briefly described.

In fig. 12 is shown an enlarging but still young prosorus. The nucleus is conspicuously large but the greater portion of it is occupied by a vacuole. The heavy nucleolus lies suspended at the centre by a few delicate linin strands, and upon the latter and upon the nuclear membrane are the remains of the chromatin previously discharged from the nucleolus and now undergoing solution in the vacuole. Scattered here and there in the reticulum of the cytoplasm are minute chromidia, which make their appearance only after the process of nucleolar discharge has begun, and bounding the whole is the thin, almost invisible membrane of the organism. The enlarged nucleus of the host cell is resting upon the parasite and its deep staining reaction indicates that its disorganisation is beginning. The protoplasm of the host cell is now reduced to an extremely thin layer upon the wall of the parasite. On the right-hand side the epidermal cell in contact with the host cell has followed the upward development of the latter and has subsequently become divided by transverse walls. This represents an early stage in the upward growth of the ring of epidermal cells surrounding the host cell which culminates in the formation of the rosette. A slight enlargement can be detected even in the second cell from the host cell, as the third, of which only a small portion is depicted, lies at the level of the surface of the tumour. The width of the outer wall of the host cell represents the original width of the cell when infection took place. On the left-hand side there are no rosette cells, and the wall of the host cell is straight, the explanation of both these relations lying in the fact that the adjacent cell on that side has also been infected; for it has invariably been found that when two infected cells adjoin, the common wall is kept straight by mutual pressure and the two organisms share the one rosette. Should a single uninfected cell intervene, however, its presence is sufficient to ensure the formation of an individual rosette for each host cell. The host cell figured has probably reached its full size, but the prosorus will continue to enlarge until it fills the lower portion of the cell (fig. 13). In this figure is shown a more mature prosorus where the contents are about to pass into the host cell. The point in the membrane through which the discharge will take place lies at the centre of the portion in contact with the finely granular protoplasmic disc in the upper region of the organism. The membrane itself, which has attained considerable thickness, is, in living material, clear in substance and golden in colour. It consists of two layers, the outer of which is thick and coloured, while the inner is thin and hyaline. The process of nucleolar discharge is now almost completed, and the nucleus exhibits the appearance typical of the migrating prosorus. The nucleolus rests upon the nuclear

membrane, while rising from it and stretching across the nearly homogeneously filled vacuole are a few long, distinct, linin strands. This is really an early stage of the prophase of the primary mitosis, the later phases of which will be completed when the nucleus is reconstituted after the passage of the prosorus into the upper part of the host cell has taken place. The number and size of the chromatin granules in the cytoplasm have markedly increased as the result of the solution of the chromatic substance in the nuclear vacuole, and its subsequent condensation into chromidia in the extra-nuclear region of the organism. The host protoplasm lying between the parasite and the walls of the cell exhibits the affinity for chromatin stains common to disintegrating cells. This is an early sign of the changes which finally result in the deposition of the protoplasm upon the prosorus as an epispore similar in colour and general appearance to the membrane of the parasite. Through the agency of this layer the envelope of the prosorus eventually becomes firmly welded to the lower portion of the host cell in which it lies.

The Discharge of Chromatin from the Nucleolus.

The process of nucleolar discharge sets in so early in the development that prosoral nuclei in which it has not yet begun are comparatively rare. Such a nucleus, however, is shown in fig. 14. It is quite small and its structure is simple, consisting only of a central nucleolus suspended by a few radiating linin strands in the centre of the large vacuole. Before the contents of the prosorus pass upwards in the host cell three discharges of the nucleolar substance takes place, but as the first is insignificant in amount and similar to the second in method a description will be given of the second and third only.

The Second Discharge.—The nucleolus in the stage immediately preceding the second discharge is large and spherical. It stains uniformly except for the small vacuole which is the result of the material lost in the first discharge (fig. 14*a*). This vacuole, however, usually disappears before the second discharge takes place. As a preliminary to this discharge, the chromatin is found to stain more deeply at one or two points on the surface of the nucleolus. These areas become gradually raised, and finally they are pushed up in the form of globular buds above the surface of the nucleolus (fig. 15). Their substance is then opened out, both upon the linin strands already present in the vacuole, and independently, in the form of dark threads of irregular outline interlacing with one another on the side of the nucleolus on which the globule was formed (fig. 16). In the earliest stage these jagged threads stain uniformly, and seem to be wholly composed of chromatin, but before they have been long in the vacuole a change takes place in their appearance, and their two-fold nature is revealed. All portions of the threads alike become paler as the result of the chromatin solution which sets in, but where the irregularities are large the process takes longer, and the narrower portions of the thread in consequence may be in an almost colourless state, while the irregularities

are reduced only to condition of isolated granules and still retain a certain proportion of their chromatin. After partial solution, therefore, the effect produced is that of linin strands beaded with minute chromatin granules (fig. 17). There is wide variation in the amount of nucleolar substance emitted at this discharge. It usually passes out in one large globule, but several smaller ones may be given off at the same time. As the substance of each is distributed the nucleolus is left with vacuoles corresponding in number and size to the globules emitted (figs. 18*a*, *b*). But, apart from the major periodic discharges, when the organism is in its normal state of active growth the nucleolus continues to give off small independent globules, so that when the first discharge has once begun it is exceptional to find a nucleolus without globules upon its surface in some stage of formation. Thus, in fig. 16, the second discharge has only just taken place and the globules upon the surface are of the occasional nature described above.

As the chromatic constituent of the threads disappears the achromatic matrix upon which it was supported is gradually revealed. As time passes the now colourless strands are widened, their edges become diffuse, and the whole substance is gradually distributed more or less evenly through the vacuole as an extremely fine network. The longer the period between this discharge and the next the more uniform is the distribution of the linin matrix through the vacuole. Two stages in the process may be seen in the nuclei of figs. 18*a* and 18*b*, and in the first in addition the linin may be traced to its point of attachment to the mouth of the large vacuole in the nucleolus. The size of the nucleus has so far been regularly increasing, but the maximum is attained during this discharge when the diameter of the nucleus may be as great as 25μ. After this stage, however, the nucleus gradually contracts until at the migration of the prosorus the diameter may not be more than about two-thirds of the maximum size.

The Third Discharge.—In an active nucleus the loss of nucleolar substance due to the second discharge will be replaced by fresh chromatic material before the next discharge begins, and the globules of the latter will once more develop upon a uniformly dark nucleolus (fig. 19). In other cases the globules are formed upon a vacuolated nucleolus, and this condition is associated with incompletely dissolved chromatin in the vacuole (fig. 20). In the nucleus of this figure the numerous globules characteristic of the third discharge have already been formed, and are now about to be discharged. Their dark colour presents a sharp contrast to the almost colourless nucleolus upon which they rest, and the latter has apparently given up the greater portion of its chromatin for their formation. During the accumulation of chromatin substance for the formation of the globules of the third discharge the nucleolus moves from its central position to the nuclear membrane.

In fig. 21 the third discharge is in actual progress and the chromatic substance is being transferred from the globules to long heavy strands. It is in a stage such as this that the function of the globules as distributing centres may be most clearly

recognised. The surface view of a nucleolus at the same stage is shown under higher magnification in section (α) of the same figure, and it will be seen that, although the nucleolus is honeycombed with vacuoles and contains very little chromatin, the globules have suffered no reduction in concentration but only in size. In the homogeneous nucleoli of the younger prosori the substance of the globule is usually drawn from the peripheral region, and the size of the resulting vacuole does not greatly exceed that of the globule formed, but in the older, vacuolated nucleoli, in addition to the granules rising near the surface, others are formed deep within the body of the nucleolus. These apparently pass up to the surface through the connecting system of vacuoles, and there either unite their substance with that of the peripheral granules or give it off independently into the vacuole. Owing to the weak concentration of the chromatic element in the old nucleolus, a large area is drawn upon in the production of a granule of the requisite density, and the relative sizes of granule and vacuole in consequence present a marked contrast to those of the younger nucleoli (*cp.* figs. 26a and 26b).

It is a characteristic feature of the third discharge that the chromatic threads in which the nucleolar substance is distributed in the vacuole are not arranged in an irregular interwoven fashion as in the second discharge, but are limited in number and extend in more or less regular sweeping curves from the nucleolus across the nucleus to the surface of the membrane opposite (fig. 21). By this time the linin arising from the previous discharge is so evenly distributed through the nuclear cavity that trace of the reticulum is almost completely lost, and in the figure mentioned only in a small region near the periphery at the left-hand side can the reticulate arrangement still be seen. In the final condition the whole background of the vacuole consists of homogeneous linin, and no reticulum remains. At this stage the nucleolus sometimes becomes flattened into a disc-shaped structure, and is closely adpressed to the membrane (fig. 23), but this is by no means an invariable rule, the original shape being frequently retained to the end.

The solution of the chromatin, perhaps owing to the altered condition of the vacuole, proceeds slowly after this discharge, and, in consequence, the method of transference from globule to strand, and the stages of solution when upon the strand can be followed more easily in this last discharge. Fig. 22 shows a small portion of a nucleolus, and at the surface is a globule which is transferring its substance to the two linin threads attached. As will be seen from the isolated positions of the granules already upon the threads solution has commenced, but transference of the substance is still proceeding, and under natural conditions it would have continued until the supply was exhausted. As the nucleus matures the width of the strands is gradually reduced, the unevennesses upon their surface are lessened and eventually removed, and they become uniformly slender and regular. In the latest stage, when the chromatin has completely disappeared from them, they persist as delicate but perfectly distinct strands spanning the nucleus.

In the early stage of the discharge, in addition to the long strands, there are frequently a number of shorter ones, but these in time disappear, and finally only five long ones remain (fig. 24). In the nucleus reconstituted after the migration of the prosorus the nucleolus is small and homogeneously dark (fig. 34). The contraction in volume, with the concentration of chromatic substance it involves, may take place before or during the migration. A peculiarity of the third discharge is that the larger irregularities upon the threads do not always remain attached to them during their solution, but frequently become separate, and then are to be seen as dark isolated granules lying usually near the periphery of the nucleus. This severing of portions of the chromatic element from the strands is probably largely responsible for the eventual smoothness and delicacy of the latter (fig. 24*a*). In this figure, contraction of the nucleolus has already occurred, although the movement, which has begun in the cytoplasm of the parasite, has not yet been communicated to the nucleus.

In the majority of cases under normal conditions the nucleus slowly undergoes these successive changes, and reaches and duly completes the final stage before the migration of the protoplasm occurs. But the description given above of the final appearance of the nucleus holds only for the usual cases in which delay has occurred between the second and third discharges, and in which the stages of the latter discharge are actually completed. At the completion of the second discharge, however, the prosorus is extremely sensitive to variation in external conditions, and its behaviour varies widely according to the regularity of the water supply in particular. Under the abnormal temperature of the greenhouse, when there is a constant supply of water, there may be no delay between the discharges, the stages of the third may be quickly passed over, and the prosorus may migrate before this discharge is actually completed. In this case, the outer envelope of the organism will be comparatively thin and still pale in colour when migration takes place. The nucleus of a prosorus which has undergone this rapid development is shown in fig. 25. The absence of delay between the discharges and rapidity in the carrying out of the third may be deduced from the fact that the linin formed in the earlier discharge has not yet completely changed from the interwoven to the reticulate condition, much less has it acquired the final uniform appearance found in prosori developed at a lower temperature. Moreover, the later stages of the third discharge will not be finished either slowly or completely before the nucleus begins to move, for the cytoplasm of the organism is already commencing to migrate (not shown in the figure). This, however, is a case of exceptionally rapid development. As a rule, a distinct pause occurs between the two discharges, and the envelope of the organism at the same time becomes thick and deep yellow in colour. Then, once the third discharge has begun, the prosorus migrates, either early or late according to the water supply, and the appearance of the nucleus at the time of migration thus depends upon the same regulatory factor.

The Passage of the Prosoral Contents into the Host Cell.

Shortly before the passage of the prosorus begins, the protoplasm immediately beneath the place where the channel will be formed becomes conspicuously dense, its vacuoles disappear, and its chromatin granules are dissolved (fig. 27). In material which has been fixed in FLEMMING'S solution and left unbleached, this portion alone of the cytoplasm appears light in colour and free from granules, standing out in sharp contrast to the mass of vacuoles deeply stained with osmic acid that masks the cytoplasm elsewhere. The protoplasm round the upper portion of the nucleus also gradually becomes condensed, and forms a small triangular cap with the apex pointing in the direction in which the discharge will take place. The cytoplasm at the centre of the superficial dense area, while still covered by an expanding portion of the thin inner lining of the envelope, now soon pushes as a minute projection through the heavy outer membrane into the clear space of the host cell beyond (fig. 28*a*). Except for the small portion of the inner lining of the envelope which lay beneath the pore, and which continues to expand as the mass of the prosorus emerges, the inner lining as a whole remains in its original position within the envelope. Under the pressure of the out-flowing protoplasm the edge of the envelope at the pore turns first up, and then slightly back as the contents expand over the free surface of the envelope (figs. 28*a*, *b*). When the projection is still quite small, the upper portion of the nucleus begins to elongate, but the basal region remains for a little time practically unaltered so that the nucleus becomes pyriform in shape (fig. 30). As the apex of the nucleus extends upwards it is preceded by an elongated cytoplasmic strand, which reaches towards the pore and consists of the substance of the cap previously surmounting the nucleus. The preliminary stage of migration takes a comparatively long time, but once the nucleus begins to move, the discharge becomes more rapid, and the passage of the nucleus itself is quickly accomplished. The elongated apex of the nucleus continues to be drawn out until it reaches and passes through the pore, but as the major portion has probably not yet moved from the original position, the nucleus at this stage may be extended over more than half the width of the prosorus (fig. 31). During this elongation the nucleolus usually lies in the posterior end of the nucleus, but it may lie further forward, and, in exceptional cases, it has been found in the narrow anterior region. In most migrating nuclei it is a conspicuous object. It may be already reduced in size, and its contents condensed and darkly stained. More often, however, it is large, has many vacuoles, and stains lightly. If it lies in the posterior end of the nucleus, the fine linin threads, which are usually five in number, will be attached to it at one end, and remain extended before it in long sweeping curves during the passage. When the nucleolus is placed in the anterior end, however, the linin threads are drawn after it. Probably a few chromatin granules, which have previously been detached from the linin threads, are to be seen on the inner surface of the nuclear membrane, and sometimes they undergo

budding during and just after the passage of the nucleus. The vacuolar region is completely filled with the fine homogeneous substance which arose as the dissolution-product of the material of the nucleolar discharges. The nucleus narrows before reaching the pore, and becomes still more reduced in diameter as it passes through it. The vacuolated condition of the nuclear cavity is never retained after the reconstitution of the normal form of the nucleus.

As soon as the pointed apex of the nucleus has emerged from the aperture of the pore it begins to swell into a spherical shape, but at the same time it continues to move forward (fig. 32). The direction of movement is maintained in the same straight line, and the nucleus finally takes up its position close beneath the thin membrane of the prosorus at a point opposite the pore (fig. 34); the last stages of the nuclear passage (fig. 33) are completed extremely rapidly, but one or two cases have been found in which, while the major portion of the nucleus has resumed its spherical form, the posterior region is still drawn out towards the pore behind it.

At the beginning of the migration of the prosorus large vacuoles arise at the periphery of the protoplasm in the original envelope as it retreats from the membrane (fig. 34). As the body continues to emerge from the pore the vacuoles become larger and larger until finally there remain only a few delicate strands which reach across the width of the envelope and unite at the pore (fig. 35). Stages in the retreat of the protoplasm are shown in fig. 36*a–c*. After the completion of the passage, and even later when several nuclear divisions have taken place, a few odd strands of protoplasm may still be detected in the vacated envelope. The migration is apparently brought about by a rapid absorption of water, and the consequent formation of a series of vacuoles round the periphery of the prosorus under the pressure of which the contents are extruded through the envelope. It is not unusual at the completion of the discharge to find a large vacuole extending from the nearly empty envelope to the centre of the extruded mass of cytoplasm (fig. 35). The vacuole there soon becomes broken up into numerous smaller vacuoles, and they in time are evenly distributed throughout the prosorus, and are then no longer distinguishable from the normal vacuoles of the cytoplasm.

Mitosis in the Prosorus and Sorus.

A considerable amount of work has already been published upon the method of nuclear division in the prosorus and sorus of other species of *Synchytrium*. *S. taraxaci* and *S. decipiens* are the two which have been frequently investigated, but even in their case investigators are agreed neither upon the universality of the occurrence of mitosis nor upon the details of such nuclear divisions, whether amitotic or mitotic, as they describe. All the species, whose nuclear phenomena have been carefully examined, belong to the group *Eusynchytrium*, to which group *S. endobioticum* also belongs. In spite of the disparity in the results so far obtained, it seems not improbable that in the prosorus and sorus not only in the group *Eusynchytrium*, but

in that of *Pycnochytrium* also, further research will reveal the existence of a uniform method of nuclear division and confirm the growing belief that that method is mitotic.

The first worker to examine the phenomena of nuclear division in the sorus was Dangeard (6), who, in 1890, stated that in *Synchytrium taraxaci* the primary division (*i.e.*, of the original nucleus, in contrast to the secondary or succeeding divisions) was always amitotic, but that at least some of the succeeding divisions were definitely mitotic. His work was confirmed by that of Rosen (37), who believed that amitosis occurred exclusively in the earlier divisions while typical mitosis was gradually evolved during the later divisions. F. L. and A. C. Stevens (46), working together upon the American species *S. decipiens*, came to the conclusions which, on the whole, were at variance with those of Dangeard and Rosen. Mitosis they maintain is the normal form of the primary division, but they qualify their statements by adding: "We may here indicate, also, that in affirming that the primary division may be mitotic, we in no way set aside the possibility of its being sometimes, even frequently, amitotic." Of the method occurring in the succeeding divisions they give no information, but Griggs (11), using their material, concluded that in these divisions both mitosis and amitosis were to be found. The cases of amitosis occurred in the divisions that immediately succeeded the primary, and two forms of it were recognised—nuclear gemmation and heteroschisis—but later in the development of the sorus mitosis was found to be the only method of division. The nuclei which arose by amitotic division Griggs believed could afterwards undergo mitosis, but no variation was detected in the number of chromosomes of the later divisions. The interpolation of amitotic division did not apparently present any difficulty to him in connection with the question of heredity, for he adds, "there is no morphological or material continuity of the chromosomes from generation to generation of nuclei; but the chromosome number is a physiological constant, like the other hereditary characters of the species."

Kusano (22), in an excellent paper upon the Japanese species *S. puerariæ*, maintained that normally all the divisions, primary and secondary alike, are mitotic, but he admitted he had at times found clusters of nuclei adhering together which he believed had probably arisen through some process of amitosis. The presence in the sorus of nuclei of unequal size has been noticed by all the writers and in every case their origin is attributed to a previous occurrence of amitosis.

In the present species Percival (35) has seen and figured the metaphase of small secondary nuclei, but though he failed to find the primary nucleus in division he was evidently inclined to the opinion that the method was amitotic for he states: "No primary nucleus was found to undergo recognisable mitotic division, but undoubted mitosis occurs in the minute secondary nuclei. In this respect my observations upon this organism agree with those of Dangeard (6) and Rosen (37) who noted the gradual evolution of typical mitosis in the later divisions of *Synchytrium taraxaci*."

Bally (1) described the secondary divisions in *S. taraxaci* as definitely mitotic, and although he did not succeed in finding the primary division he concluded that it also was mitotic. In the primary nucleus, however, he describes a passage of chromatin granules from the nuclear vacuole into the cytoplasm, which if confirmed, would but add another form of amitosis to the numerous types already described for *S. taraxaci* alone.

With the publication of a paper upon *S. taraxaci* by Rytz (38) in 1917, however, the aspect of the question of nuclear division in that species was completely changed. In this investigation it is maintained that mitosis is the only method of division of both the primary and the secondary nuclei.

All the investigators who succeeded in finding cases of mitosis agree that the spindle is intranuclear in origin and that prior to the formation of the chromosomes a large portion of the substance of the nucleolus is given off into the nuclear vacuole. Concerning the time of the disappearance of the nuclear membrane, however, there is wide variation of opinion.

Thus in spite of the thirty years which have elapsed since Dangeard's paper was published, no definite conclusion has been formed concerning the nature of either the primary or the secondary divisions. On the whole, however, mitosis has come to be regarded as the normal method, and the accepted range of its occurrence has been extended to include the division of the primary nucleus, while concomitantly amitosis is now considered a somewhat exceptional method of nuclear multiplication in the genus and ranks as a subsidiary phenomenon only.

The Division of the Primary Nucleus.

Prophase.—Although the beginning of the prophase of the first nuclear division is difficult to determine, it may be considered to begin with the setting in of the processes of the third (last) nucleolar discharge, as it is at that time that the changes are initiated that lead directly to the formation of the spindle and chromosomes. By the time migration of the organism commences the surplus substance of the nucleolus has been given off, the vacuolar region is in the dense homogeneous condition characteristic of it during metaphase and anaphase, and the fine linin threads which will constitute the spindle are already formed. Actually there is no break between the pre-migration and post-migration stages of the nucleus. The migration of the whole prosorus may be completed in four hours; the nucleus moves early, and by the time two-thirds of the cytoplasm have passed from the envolope, it will have reached its final position in the extruded portion, have regained its original shape, and be preparing for the formation of the spindle of the primary division (fig. 34). Two nuclear divisions usually occur before the cytoplasm has passed out completely, that is, since the rate of movement of the second half is more rapid than that of the first, in less than two hours (fig. 35). If the early divisions in other species of *Synchytrium*

take place at a similar pace it is not strange that difficulty has been experienced in securing stages of the primary division.

The appearance of the reconstituted nucleus closely resembles that of the nucleus before migration, except that the former is slightly smaller (fig. 34). It is oval in shape and lies close to the free surface of the prosorus with its longer axis at right angles to the direction of migration. The cap of dense cytoplasm which lay upon its upper surface before migration and passed in advance of it when movement began, becomes spread out close to the surface of the prosorus in the neighbourhood of the nucleus. The lower side of the nucleus is sometimes slightly flattened, perhaps owing to the pressure of the cytoplasm which is still flowing up beneath it. The nucleolus lies in an excentric position close to one of the ends of the nucleus. It is reduced in size and always stains deeply. The free chromatin granules sometimes visible in the nucleus at the beginning of migration have usually disappeared by the time it takes up its final position. The nuclear membrane is extremely delicate, but it separates sharply the fine homogeneous ground-substance of the nucleus from the somewhat dense, non-vacuolated cytoplasm immediately surrounding it.

After the reconstitution of the nucleus one or two buds appear upon the surface of the nucleolus, and it is believed that the chromosomes are formed from one of these on its separation from the nucleolus (figs. 39, 40). The five linin threads which before and during migration extended in long open curves from the nucleolus are now contracted somewhat towards the latter and form a loose coil of interwoven threads, the individual strands of which, however, remain distinct (fig. 37). This stage soon passes and they are drawn out again until they lie close, and almost parallel, to one another through the agency of extremely fine, only just visible linin threads, which in specially favourable material may be seen attached to ends of the strands, joining them to the nuclear membrane (figs. 38, 39). The nucleolus now becomes slightly separated from the strands, and they become finer and finer the nearer they approach one another (fig. 40). At the stage when they are almost touching to form the spindle five minute oval chromosomes can be detected upon them in the equatorial region. The actual origin of the chromosomes was not observed, but as the nucleolus persists intact but reduced in size after the formation of the spindle, and, as in addition, no free chromatin granules (save occasional disintregrating particles on the inner surface of the membrane) can be found in the nucleus at the time, the conclusion has been drawn that the chromosomes originate probably from one of the latest nucleolar buds given off after the reconstitution of the nucleus.

F. L. and A. C. Stevens (46) believed that in *S. decipiens* the substance of globules found in the nuclear vacuole was drawn out into sharply intersecting, short linin threads, which later coalesced, to form the strands of the spindle. The dense halo of granular substance noticed round the latter at metaphase was attributed to a swelling and gelatinisation of the nuclear membrane, and the possibility of its being the after-product of an intranuclear element, as in *S. endobioticum*, was not con-

sidered. The chromosomes, which they figure as long narrow structures, were said to be only four in number, in contrast to the five of the present species. The only other description of a primary mitosis is that given by KUSANO (22) for *S. Puerariæ*. In this species, the dense background surrounding the newly formed spindle was believed to represent a dissolution product of the nuclear elements. In the case of *S. endobioticum*, however, we may go a step further, and state that the element in question is the linin residuum of the chromato-linin globules discharged from the nucleolus, and that in many cases it is a conspicuous feature some time before the spindle is formed. The spindle itself, KUSANO believes, is usually constituted from the residue of the nucleolus, but he gives no explanation of its origin in those cases in which the nucleolus was found to persist throughout the later stages of mitosis. The chromosome number is stated to be definitely five, and thus it agrees with that of the present species. In one particular, however, that of the time of the disappearance of the nuclear membrane, there is not complete accord between KUSANO'S description and the behaviour of *S. endobioticum*. In the former it disappears before any sign of spindle formation is to be detected, whereas in the latter it persists until metaphase—a condition which KUSANO acknowledges to be normal for all succeeding divisions in *S. Puerariæ*.

Metaphase.—The spindle is extremely long and slender, and stretches across the full length of the nucleus (fig. 41). The latter becomes slightly elongated in the direction of the spindle, and the nuclear membrane thereupon quickly disappears. The nucleolus is now much reduced in size and lies near the periphery of the nucleus at some distance from the spindle. A few disintegrating chromatin granules may still be seen near the junction of the ground substance of the nucleus and the surrounding cytoplasm. As the nuclear membrane disappears, nucleolus and granules pass out, and become lost to view among the numerous dark granules of the cytoplasm. No swelling of the membrane prior to its disappearance, such as STEVENS reported, occurs in this species. During prophase it is extremely delicate, but, until metaphase is established, it remains sharply differentiated. When the large size of the original nucleolus, and even of the remnant that persists at metaphase, is considered, the chromosomes are unexpectedly minute. Their form is almost spherical, and they lie close to one another in the equatorial region of the slender pointed spindle. The cytoplasm, on the disappearance of the nuclear membrane, has been stated by previous writers to "encroach upon" the nuclear area. The sharpness of the distinction between the two regions in the present species is admittedly gradually lost, but, even at the completion of the second mitosis, the limits of the primary nucleus may still be recognised. The eventual uniformity in the structure of the protoplasm seems to be secured rather by an opening up of the portion which is of nuclear origin by progressive vacuolisation working from the periphery inwards than by an actual advance of the cytoplasmic region into the area previously occupied by the nucleus.

Anaphase.—As the daughter chromosomes separate and pass to the poles, the individual members of either group maintain their relative distance apart for almost the whole length of the spindle, and only as they approach the poles do they exhibit a tendency to come together. In the region of the spindle lying between the separating groups the fibres remain parallel until the chromosomes have nearly reached the poles, when elongation of the spindle and a narrowing of the central region begin. The spindle, to begin with, is long, and it is customary in the primary division for only a slight further elongation to take place, so that sometimes the daughter nuclei are formed within the original nuclear plasm. The latter follows the spindle in such elongation as it undergoes, and constitutes the "halo" described for other species. No trace of centrosomes or polar radiations could be detected.

The telophase of the primary mitosis was not found.

The Division of the Secondary Nuclei.

Resting Stage.—The secondary nuclei, even those which result from the first few divisions, are unexpectedly small. The nucleolus remains conspicuous in the successive generations of nuclei until the sporangia approach zoospore formation, by which time the nuclei are extremely minute, and contain only two or three chromatin granules, so similar in size that a distinct nucleolus can no longer be recognised (figs. 42, 62). The linin in the nucleus occurs as slender strands, and, until prophase commences, the nuclear vacuole is comparatively free from the anastomosing threads, such as those which constitute the reticulum in the maturing primary nucleus. The nucleolus, in addition, is excentric in position in the secondary nuclei. In the case of *S. Taraxaci*, Bally (1) considered it improbable that the chromatin granules of the secondary nuclei were derived from the nucleolus in the same way as those of the primary nucleus. Their size, which is large when compared with that of the nucleolus, and the absence of a linin reticulum, which, being a product of the nucleolar discharge would confirm the previous occurrence of a nucleolar discharge, seem to militate against the possibility of a parallel method of derivation of the granules. However, the frequent occurrence of chromatic globules upon the surface of the nucleolus so closely resembles the accumulation of chromatin granules on the nucleolus of the primary nucleus that the origin of at least a certain proportion of the chromatin granules of the secondary nuclei may safely be said to be nucleolar. As it is probable that the method of production is uniform for all the granules, it has been concluded that in *S. endobioticum* at least, the chromatin granules, as a whole, probably owe their origin to some form of nucleolar discharge.

Prophase.—At the beginning of prophase, the chromatin granules on the nuclear membrane stain less deeply and diminish in size, and, as they undergo solution, a linin reticulum makes its appearance across the vacuole of the nucleus (fig. 43). The nucleolus buds freely and the granules formed travel to the periphery, and in turn they, too, fade and disappear (fig. 44). During this process the final density of

the linin reticulum is attained, and the vacuole, as in the prophase of the primary division, thereby becomes filled with a homogeneous substance. As in the latter also, the fine linin strands, which will enter into the constitution of the spindle, may be seen at this stage attached to the nucleolus, and extending from it in sweeping curves to the nuclear membrane (fig. 45). When the nucleus has become almost free from granules, a globule is given off from the nucleolus, and at times it is almost as large as the remainder of the nucleolus (figs. 46, *a*, *b*, *c*). The chromatin of this globule becomes concentrated in the free end as it separates from the nucleolus, and it is believed that it is from this that the five chromosomes are formed (fig. 47). When the difference in size between the primary and the secondary nuclei is considered, a corresponding inequality might be expected in the size of their respective chromosomes. It is not more pronounced, however, than is that between the chromosomes of secondary nuclei of different generations.

Metaphase.—The spindle of the secondary nucleus, when compared with that of the primary, is short, relatively broad, and ends abruptly in a sharp point immediately within the nuclear membrane (fig. 48). The nucleolus at metaphase may be seen as a distinct granule lying between spindle and membrane, and it constitutes the only free chromatin within the nucleus. Upon the disappearance of the membrane, slight elongation of the spindle sets in before the chromosomes divide, and the nucleolus at the same time passes to the periphery of the nuclear region (figs. 49, *a*, *b*). Prior to division the chromosomes elongate and the daughter chromosomes then appear as minute spherical granules (fig. 50).

Anaphase.—As in the primary mitosis, each chromosome maintains its distance from the other members of its group during the passage to the poles (figs. 51, *a*, *b*). The final elongation of the spindle varies, but on the whole it is not as great as that found by BALLY (1) in *S. Taraxaci*. Where it is slight it is often accompanied by an extension of the nuclear plasm, but as the chromosomes approach one another near the poles they pass beyond the limit of this plasm (fig. 51 *b*).

At *telophase* the chromosomes unite to form a darkly staining globule, in which the end of the spindle seems to be included (figs. 52, *a*, *b*). The spindle, which until late anaphase was maintained at the full width of the equatorial region at metaphase, is drawn out at telophase into a thin, dark, curved strand, connecting the daughter nuclei. A vacuole, which quickly enlarges to a conspicuous size, then appears round the chromosomes as they unite to form the nucleolus (fig. 53). A few linin threads make their appearance, and the nucleolus, after reaching a certain size, begins to give off portions of its substance as chromatin globules. Several globules are usually extruded at one time, and each nucleus as it matures normally undergoes two or three periods of nucleolar budding in rapid succession, until eventually the resting stage, with its numerous chromatin granules, is attained. In figs. 54 and 55 are depicted almost parallel stages in the evolution of the resting condition of large and of small nuclei respectively. The general method of development is the same

whatever the size of the nucleus. Different stages in the budding of the nucleolus for the formation of the granules are to be seen in fig. 54 *b*, and figs. 55, *b*, *c*. No trace of centrosomes or asters was found, but since in the two species in which their occurrence has been reported they are present during only a short portion of telophase, it is possible that suitable stages have not yet been obtained in *S. endobioticum*.

Before the segmentation of the prosorus into sporangia the nuclei after each division regain almost their original size before again dividing, but after segmentation has taken place there is a noticeable difference in the size of the nuclei of succeeding generations, and when a few further divisions have occurred they are reduced to the minute nuclei of the zoospores.

The extra-nuclear chromatic granules which from an early stage are conspicuous in the cytoplasm of the parasite begin to disappear as nuclear division sets in. They are still quite distinct, however, at the time of segmentation, and only when the sporangia are nearing the formation of the zoospores do they completely vanish (fig. 62). Both PERCIVAL (35) and BALLY (1) describe a frequent occurrence of nuclei of unequal size in the prosorus of *S. endobioticum*. In spite of an exhaustive search, their results could not be confirmed, for no unequal nuclei were observed in the prosorus. In the sorus, however, one sporangium was seen which was mature and just forming its zoospores, and which possessed a large nucleus, differing widely in size from the three hundred other normal nuclei. But this was a case of arrested development and not of unequal rapidity of division. Moreover, its occurrence was unique. BALLY (1) suggests that the inequality of what he believes to be nuclei is possibly to be referred to amitotic division. Any method of division other than mitosis, however, certainly does not occur in either prosorus or sorus. In the illustrations given by these writers in which large nuclei and somewhat smaller nuclei are shown, the apparent difference in size would seem to be explained by differences in the angle at which nuclei of equal size were cut. Where the difference in size is very much greater, however, it seems probable that the chromatic granules of the cytoplasm have been mistaken for nuclei. This suggestion is supported by the large size of the normal nuclei in each figure, for at the stage at which such nuclei are present in the parasite granules are always numerous in the cytoplasm. From the present work it has been concluded that mitosis is the sole method of nuclear division in both prosorus and sorus, that inequality in the size of the nuclei is extremely rare, and that when it does occur it is probably abnormal.

The Segmentation of the Prosorus.

There are at present two theories concerning the method of segmentation in the genus. The earlier, which was proposed by HARPER (15), and has since been widely accepted, supposes that a shrinkage of the protoplasm of the prosorus occurs, that water is given off, and that invagination of the membrane of the body at several

points on the surface then follows. The channels of separation thus formed progress inwards, and finally meet at the centre, thereby cutting the body into multinucleate segments. These segments may be further subdivided into uninucleate "protospores," or develop from their multinucleate condition without subdivision, according to the species. Until KUSANO'S paper appeared, this theory was largely held, and KUSANO (22) himself believes that some prosori divide by a somewhat similar process. Nevertheless, for other prosori, he describes a method of segmentation in which the preliminary shrinkage is omitted, while, in place of the cleavage channels of the invagination theory, cleavage membranes are formed between the nuclei, although independently of their division.

In *S. endobioticum*, the prosorus, at the time of cleavage, contains about thirty-two nuclei, and, as they are distributed evenly through the cytoplasm, the number included in each segment varies according to its size. The walls appear simultaneously throughout the organism. Their formation takes place independently of nuclear division, although it often begins when the nuclei are in early telophase, in which case it is completed before they again divide. There are usually not fewer than four sporangia in a sorus, while there may be as many as nine, but five and seven are the numbers most frequently found (fig. 60). In two exceptional cases, division was omitted altogether, the prosorus giving rise directly to zoospores.

The first indication of the approach of segmentation is given by the protoplasm becoming slightly denser, and the size of its vacuoles diminishing, along the course of the future walls (fig. 56). In the middle of these areas, an especially deeply-staining layer is differentiated, which, before it is sharply delimited, follows a minutely undulating path as it skirts the vacuoles (fig. 57). The layer then shrinks in width and becomes less undulating, while in the middle of it there is differentiated a delicate, clearly defined membrane (fig. 58). As the latter becomes firmer, the vacuoles in the adjacent protoplasm gradually regain their normal size. When fully formed, the walls intersect one another at wide but definite angles (fig. 59). During the last few years, somewhat undue stress has been placed upon the shape of the sporangia when enclosed in the sorus—whether their surfaces are sharply angled and fit together, or rounded and merely adjacent—but both these relations are exhibited by all sporangia in the normal course of development. When young they fit together, as the membrane of separation between any two was originally common to both, but, as they enlarge, they naturally become rounded, although mutual pressure may keep the surfaces in contact still flat. The shape of the sporangium is thus largely dependent upon the accident of spatial limitations, and it should not be regarded as a character of diagnostic value, as it has been in several instances.

The Sorus.

General Development of the Sporangia.

After the formation of the sporangial walls, the nuclei divide repeatedly, and decrease in size at each division, until the two to three hundred minute nuclei of the zoospores are formed (figs. 60–62). Mitosis takes place simultaneously throughout each sporangium, but is independent of the process in the other sporangia of the sorus. The cleavage walls, as a rule, are formed during the telophase of the prosoral nuclei, and this phase is usually completed simultaneously in all the sporangia. Intersporangial variation is begun owing to the different durations of the ensuing resting period. A difference of a complete generation may be introduced at the next division through the nuclei of only a proportion of the sporangia participating in it (fig. 60). But gradual variation, resulting at maturation of the sorus in a more or less regular series of nuclear stages, is, on the whole, more common. The final and maximum difference exhibited by the sporangia of any sorus has not been found to exceed that of two nuclear generations, and usually it is considerably less. In one mature sorus observed, five out of the total seven sporangia were at exactly the same state of development, but this was an exceptional case. Fig. 61 shows a sorus with three sporangia whose nuclei are at the same stage.

The substance of the extra-nuclear chromatin granules decreases in amount during the further development of the enlarging sporangium, and finally they disappear. At the same time, the protoplasmic vacuoles, which are conspicuous during the prosoral and early soral stages, become much reduced in size. A portion of a sporangium, shortly before the delimitation of the zoospores, is shown in fig. 62. The nuclei are numerous and small. Their chromatin granules are almost equal in size, and lie upon the nuclear membrane, while joining them here and there are short linin strands. No granules are to be detected in the protoplasm. Occasionally, the sporangia are liberated at this stage, and the formation of the zoospores then takes place as they lie free upon the surface of the tumour, or, if the sporangia have not been completely wetted, as they float upon the water which was the cause of the rupture of the host cell. But usually before the sporangia are liberated, the zoospores are at least delimited even if they are not completely separated.

The Formation of the Zoospores.

Before the formation of the zoospores begins water must be absorbed in considerable quantities by the sporangium. The general appearance of the protoplasm then becomes lighter and on careful examination it is evident that the somewhat transparent effect is the result of slight vacuolation of the protoplasm in a small region immediately surrounding each nucleus. Separating the vacuolated areas, and intersecting one another roughly at right angles, are short strands of protoplasm which

have retained their original density, and by the combined intersections of which the delimitation of the zoospore areas is effected (fig. 63). In each area, lying at the edge of one of the delimiting strands a single chromatin granule can be detected, and in well stained preparations a dark but delicate thread is also visible connecting it to the nucleus (fig. 63). This granule is the blepharoplast, and both it and its connecting thread can be readily detected in the zoospore in all subsequent stages until fusion or entry into the host plant takes place. Of the origin of the blepharoplast little can be said. It is usually held that it is to be traced to structures present in the spindle of the preceding mitosis. In the present case, however, nuclei at the resting stage have been seen which had not formed blepharoplasts and which yet were apparently of the same size as those of the zoospores; but it is difficult to secure thoroughly satisfactorily fixation of the sporangium at this stage and the slightest contraction is immediately reflected in a reduction in the size of the nucleus. The smallness of the nuclei in the last few generations moreover, renders somewhat insecure the assumption that any particular nucleus belongs to the last generation.

As the sporangium continues to absorb water minute vacuoles make their appearance in the centre of the delimiting strands. They gradually increase in size and the young zoospores are forced slightly apart, although for some time each still remains connected to its neighbours by fine threads of protoplasm (fig. 64). As separation continues variation occurs in the length of these connecting threads (fig. 65). Attached to each zoospore is one thread which becomes much longer than the others and continues to increase in length after the shorter ones disappear. The persistent thread is attached at one of its ends to a blepharoplast, and at the other to another zoospore (though not to its blepharoplast), or as more often happens to other similar long threads (fig. 66). As the full length is attained the threads break apart at their junction, or from the second zoospore as the case may be, and each zoospore, equipped with a single long cilium thereupon becomes free in the sporangium. No case of the direct attachment of a cilium to two blepharoplasts has been found.

The Liberation of the Sporangia.

About the time of the cleavage of the prosorus into sporangia the cells of the tissue lying beneath the host cell undergo several divisions in which the newly formed walls lie close together and parallel to the surface of the tumour, presenting an appearance not unlike that of a cambium. During the maturation of the sporangia these cells enlarge in the upward direction, and in doing so push the host cell and the rosette above the level of the surrounding surface, the epidermal cells adjoining the rosette meanwhile dividing to keep pace with the expansion (fig. 71). As the sporangia swell at maturity they exert great pressure on the host cell from within, and at the same time pressure is exerted on the host cell from without by the expanding cells of the tissue below. As the result of the two pressures the host cell bursts at the small exposed region of the outer wall between the cells of the

rosette and the sporangia, if they are not jerked out, at least lie exposed to the exterior. The enlarging cells of the host tissue below sometimes grow to an enormous size, even greater than that of the cells of the rosette (fig. 67). In *S. puerariæ* they expand still more and raise the liberated sporangia high above the surrounding host tissue, and the scattering of the sporangia of *S. fulgens* upon the surface of the host "wie lose Uredosporen," is probably to be referred to a similar process.

The Formation of the Rosette.

The young prosorus usually reaches its full size before any indication of an upward enlargement can be detected in the ring of epidermal cells in contact with the host cell. Elongation begins, however, shortly before the contents of the prosorus migrate. It may be confined to the single ring of cells in contact with the host cell, or those adjoining these may also participate, although to a smaller extent. During the enlargement of the prosorus, and later of the sorus, the rosette cells keep pace with the expansion of the host cell, but a small central area on the epidermis of the latter always remains free. The mature rosette exhibits wide variation in size and structure. The longer cells are usually divided by transverse septa, but when the rosette remains small, division may be omitted. They are remarkable for the extraordinarily rapid growth which can be induced by raising the temperature. When removed from a temperature of 16° C. and placed in an incubater at 20° C. they will elongate to twice their original length within four hours. These cells have a shining refractive appearance in the fresh state, and the cell sap appears to contain oil since it darkens with osmic acid; the blackening, however, is never so deep as that of the vacuoles in the resting sporangium or prosorus. (For stages in the development of the rosette see figs. 68–71.)

The Discharge and Structure of the Zoospores.

On the separation of the zoospores the sporangium, if covered with water, becomes clear and almost transparent. As it continues to enlarge, a hyaline projection, sometimes followed by a second or even a third, appears at one or more of the angles on the surface (fig. 72). Rupture will take place at one of these projections. If several are present, it will be at the last formed; but if only one develops, it may take place through it or through an unswollen region of the membrane. Vigorous movement of the zoospores within the sporangium has not been found to occur before at least a few zoospores have been discharged. The hole in the sporangial membrane is really a small slit. The behaviour of the zoospores at discharge depends upon their age, that is, upon the time that has elapsed since they were completely separated from one another in the sporangium. It frequently happens that sufficient water is present to secure the formation of the zoospores, but not to effect the liberation of the sporangia from the host cell. Zoospores, when fully formed, can remain unharmed in the sporangia for nearly a week, provided the sorus is not ruptured.

On the addition of water after such a period of delayed development, the zoospores are usually liberated from the sporangium simultaneously with the discharge of the latter from the host cell. When the bursting takes place suddenly in this way, the opening in the sporangium may be a slit of considerable size. The zoospores dash through it in a condition of violent activity, and it is difficult to follow the movement of any zoospore for more than a few seconds. When a few zoospores have escaped, those inside become as active as those without, and in time they too dart through the opening, and the sporangium eventually is left in a collapsed condition, with perhaps one or two zoospores caught in its folds. The most active movement, which consists of a dashing to and fro over short paths, may last from 10 to 20 minutes. Then the rate is diminished and the characteristic jerk of the cilium and body at each change in the direction of the path can be seen. In from 30 to 40 minutes movement will gradually cease and the zoospore when not on the surface of an object suitable for penetration suffers disintegration. The surface of the host tissue seems to offer an attraction to the zoospores. Again and again, when in their most active condition, they dart straight to the epidermis but fly off again immediately. As their speed is reduced, however, they may be observed to pause for a moment before returning to the open a little more slowly than they advanced. Each time they return to the host surface they stay longer, and each time they reduce the length of the backward path until finally quite 90 per cent. remain upon the plant tissue. Certain of the zoospores may swim away, but from the relations between host cells in which sori had developed and zoospores at rest on the surface, in material in which discharge of the zoospores took place under natural conditions, it has been concluded that the majority of the zoospores do not travel far from the host cell in which they develop.

On the other hand, when immature sporangia are liberated from the host cell a certain time necessarily elapses before the zoospores are discharged, the length of the period varying according to the initial state of development of the zoospores. When these sporangia rupture the hole is usually small and the zoospores slip out one at a time and remain inactive just outside the aperture. Those inside may then become active, but their movement is not so rapid as that of perfectly mature zoospores. Frequently it is only slight, and they seem to drift out rather than actively to seek the open, and they then may remain congregated in a mass outside the opening for five to ten minutes before movement begins. Sometimes, indeed, they do not become motile at all, but simply die where they lie. Usually, however, a sluggish movement sets in and they go through the same activities as the mature zoospore, although at a slower rate. Eventually they, too, come to rest upon the epidermis of the tumour, or of new potato tissue if it happens to be close at hand. The probable cause of their congregating outside the opening of the sporangium is that the cilia have not yet become free, and the zoospores in consequence are still joined together.

The activity of mature zoospores is maintained under wide variation in the external conditions. It will continue in light and in darkness, although a very bright light prevents the speed from reaching its maximum. The temperature may range from 12° C. to about 19° C. without inducing any inhibitory effect. If it is raised above 20° C., however, movement is checked. The media used for the discharge of zoospores, named in order of suitability, were rain water, conductivity water (distilled over glass), and tap water.

The zoospore, which is about 1·5 μ long, is oval in shape, but tapers slightly at its anterior end where there is a large clear refractive spot (fig. 73). The main portion of the body is slightly more dense in appearance. The cilium, which is of the characteristic chytridian length, is attached to the broader posterior end, and provided the movement is sufficiently slow can be observed in the living zoospore.

At first, difficulty was experienced in retaining zoospores upon the slide for the purpose of fixation, but eventually a simple method was evolved by which few zoospores were lost during manipulation, whether they occurred singly or were undergoing the process of fusion. The adhesive consists of equal parts of albumen and pure glycerin, and is made up each time before use. It is spread as thinly as possible by means of a second slide and not by hand, for by the latter method irregularity is introduced in the evenness of the film. It is then wiped off except for a small area in the centre of the slide about half an inch in diameter, and upon this alone the zoospores are placed. The zoospores meanwhile have been prepared in water upon a second slide, and when they are at the stage at which fixation is desired they are drawn up into a graduated tube of which the bore of the free end is no wider than a hair. To the wider end of the tube is attached about three-quarters of a yard of flexible rubber tubing and this in turn is connected to a mouthpiece, by the use of which greater freedom is left to the hands. The process of picking up the zoospores is carried out under the microscope, and they are then deposited on the prepared area of adhesive. If the number of zoospores in the first drop is insufficient, more drops may be added until a practicable number is obtained. Finally, the drop is left to evaporate until the slide can be handled without danger of spilling the drop. It is then placed for a few moments in a Roux tube containing crystals of iodine. The lower end of the tube is slightly warmed and the zoospores are killed and temporarily fixed by the fumes; fumes of osmic acid were sometimes used in place of iodine. The slide is then kept in a desiccator until the drop has evaporated, when it is placed in vapour of alcohol. On the coagulation of the film of albumen it is brought down through changes of alcohol to water, and a little Flemming's fixative (the strong solution diluted with an equal quantity of water) is placed upon the area and left there for two hours. The zoospores are now permanently fixed. Henceforth the slide may be treated as in the case of an ordinary mount, and the objects may be bleached and stained at will. For the staining of the nucleus Breinl's triple stain gives excellent results, but for that of the blepharoplast, connecting

thread, and cilium the best stain is HEIDENHAIN'S iron alum hæmatoxylin. The cilium is more sharply defined when bleaching is omitted.

The nucleus, which is conspicuous, lies in the narrow anterior end of the zoospore (fig. 74*a*), and together with one or two small fat droplets in its neighbourhood is responsible for the appearance of the large refractive spot in the living zoospore (fig. 74*b*). In *S. puerariæ*, on the other hand, KUSANO describes the position of the nucleus as central, while the fat droplets alone lie further forward. In *S. endobioticum* the nucleus is practically spherical and contains a large central vacuole. Three or four chromatin granules of about equal size rest upon the membrane, and in some cases one or two linin threads may be seen extending across the vacuole from granule to granule. Very little protoplasm is present in the zoospore. It is slightly denser in the region immediately beneath the nucleus, and from this part it extends as delicate threads round the periphery of the body, the central region being occupied by a large vacuole, which, however, does not contain oil. The blepharoplast, which is usually a little smaller than the chromatin granules of the nucleus, is to be found at the surface of the zoospore, at the end opposite that occupied by the nucleus (fig. 74*a*). The thread connecting blepharoplast and nucleus frequently lies in a strand of protoplasm, and so follows the contour of the body in a curved path.

Both zoospores and zygotes adhere firmly to the surface of the host plant once they have come to rest upon it, and every stage, from the zoospore immediately after its discharge to the zygote as it penetrates the epidermis, can readily be found on the surface of the tumour.

The Entry of the Soral Zoospore.

The zoospore, when at rest upon the epidermis of the host plant before entry, lies usually upon its side, and the nuclear end is thus raised above the surface (fig. 77); but occasionally the zoospore becomes attached by the anterior end, when it sinks slightly upon the host extending the surface of contact, and the form changes from a rounded to a low oval. The cilium now undergoes contraction. This process begins at the free end which becomes swollen, and the shortening and swelling then extend down the cilium towards the blepharoplast (figs. 75*a*, *b*), the cilium being eventually reduced to a short rod-like body (fig. 75*c*). The region at the point of attachment to the zoospore is the last to remain slender, but the substance of the cilium is eventually drawn back upon it, and a beaded darkly staining structure results (figs. 75*d*, *e*). In the last stage it is reduced to a heavily staining globule, which rests upon the surface of the zoospore near the blepharoplast (fig. 75*f*). Whether this globule is thrown off or withdrawn into the body of the zoospore cannot be definitely stated, but as many stages of zoospores in the process of penetrating the host have been found it is unlikely that the withdrawal into the zoospore of such a deeply staining body could be overlooked. In all probability, therefore, the cilium is thrown off after being contracted.

The entry of the zoospore into the host may begin soon after the cilium begins

to shorten (fig. 75*c*), but it is more often postponed until the contraction is almost complete. A change in the nucleus is the first sign that the process of entry is beginning for a projection appears on the side of the nucleus towards the epidermal surface. This projection lengthens (fig. 76*b*) and finally reaches the surface of the zoospore, where it lies in contact with the host wall. By this time the projection consists of a chromatic granule on the zoospore surface and a deeply staining thread of nuclear material, connecting the granule to the main body of the nucleus (fig. 77, where the blepharoplast is also visible). In fig. 76*a* the projection may be seen as it leaves the nucleus, while in fig. 76*b* it has advanced almost to the surface.

The earliest stage of actual penetration that has been observed shows the nuclear projection piercing the wall and extending slightly beyond into the cell. The projection, however, is so small that the thin layer of cytoplasm which doubtless covers it cannot be detected (fig. 78).

When the nucleus is passing through the pore the long heavy strand into which it is extended can often be traced from the exterior to the interior half of the zoospore without a break (fig. 79). In fig. 80 the first part of the projection has passed within the wall, and in fig. 81, which is another surface view, a small portion of the body of the zoospore has also entered. If the nucleus lies close to the surface of contact, great elongation will not occur as it approaches the pore, but usually it lies at a little distance, in which case it is drawn out into a narrow strand extending at times half-way across the zoospore (fig. 82). In fig. 83 the nucleus has almost completed its entry, while in figs. 84 and 85 it is wholly within the host cell wall, while only a vesicle of protoplasm remains outside. The last stage of entry is shown in fig. 86, where both cytoplasm and nucleus have completely entered, but the latter still remains connected to the pore, although its major portion has already resumed its spherical form. The posterior nuclear extension is soon drawn to the nucleus proper, which then becomes completely spherical. The central nuclear region does not open up to form a vacuole similar to that of the zoospore nucleus, but the granules at the periphery lie close together and merge into one another, so that the nucleus as a whole gives a deep staining reaction.

The parasite on entry represents the youngest stage of the prosorus. It soon moves from the epidermal wall of the host cell. Sometimes its route lies close against the side wall of the latter (figs. 87, 88), or it may take a more diagonal course until it approaches the host nucleus. The latter is large, and may extend almost across the cell, but there is usually sufficient space for the small parasite to pass beyond it. In the normal rapidly dividing epidermal cell, which is long and narrow, the organism passes directly to the inner end before enlarging to any extent. But in other cases enlargement may commence before it moves far from the epidermal wall, in which event it does not penetrate deeper than the neighbourhood of the nucleus (fig. 90). The protoplasm of the organism increases rapidly in amount, and is broken up into a uniform network by the formation of numerous small vacuoles.

Enlargement of the nucleus meanwhile keeps pace with that of the cytoplasm. The production of chromatin has taken place so rapidly that it now stains in a more or less homogeneous manner, and closely resembles the nucleolus of the more mature prosorus (fig. 89). At the same time a small nuclear vacuole is formed round it; this quickly enlarges to the dimension characteristic of it in later stages (fig. 90), and on the appearance of a few linin strands within it the typical nucleus of the young prosors is evolved.

The outline of the history of one complete soral generation has now been traced. It remains to follow the more normal course of events in which fusion of the zoospores takes place after the formation of the first generation of sori. For the production of several soral generations is rather a repetition of a particular phase, owing to the subjection of the organism to certain conditions at a critical period, than the normal sequence of events in the life cycle.

The Fusion of the Soral Zoospores (Gametes).

The fusing cells are exactly similar, so the process is one of isogamy. Those zoospores whose movement is sluggish from the time of their discharge have not been found to fuse. The fusion of active zoospores, however, may be watched beneath the microscope. No attempt at fusion is made when movement is at its height, but by the time the perceptible pause has been introduced at the change in the direction of the path it may be observed that two or more zoospores occasionally dart in the same direction for a few seconds. As the pace is further reduced this develops into a deliberate following of one zoospore by a second. On one occasion two zoospores from different sporangia approached each other at a steady pace. They met, then one turned and swam slowly away at an angle to its original direction. The second swam after the first at an equal pace, following immediately the five or six alterations in the path of the leading zoospore which were observed during about half a minute. Then the second zoospore stopped, whereupon the leader turned and came back, and after gliding along the stationary zoospore once or twice settled beside it, and in time the two fused. Frequently zoospores meet, remain together for a moment, and then separate rapidly without exhibiting any further tendency to fuse. Fusion is usually effected between a pair of zoospores, one of which has already come to rest before the second apparently by accident slowly swims up, touches it and, as if as the result of contact, loses its activity. It may, however, be seen to move along the surface of the stationary zoospore before finally coming to rest. When fresh zoospores join a stationary group the pairs are frequently rearranged, and some of the original members may swim away.

The Conditions Necessary for Fusion.

It is believed that fusion does not occur between individuals originating from the same sporangium, for whenever isolated ruptured sporangia were found on the surface

of the tumour only zoospores were to be seen in their neighbourhood; whereas when the remains of several ruptured sporangia lay near one another, zygotes were present in their vicinity. Fusion, however, can take place between zoospores originating from sporangia of the same sorus, for zygotes are frequently to be seen at the base of ruptured host cells when no other sori lie near; but as the sporangia are usually at different stages of development when liberated from the host cell, the zoospores will probably not be discharged at one time, in consequence of which the fusion of such nearly related gametes is possibly not of frequent occurrence under natural conditions. It is not usual for more than five nuclear divisions to occur between the segmentation of the prosorus and the formation of the zoospores. In the event of the fusion of gametes from the same sorus the nuclei will therefore probably have developed independently for not more than five generations. It is possible that independent nuclear development may have originated further back in the multinucleate prosoral stage but of that we can have no proof.

One exceptional case observed is worthy of mention. A large sporangium in an unruptured sorus contained from 500 to 600 zoospores. The tissue in the neighbourhood was decaying, and the cells adjoining the host cell were already dead. In addition to the zoospores, the sporangium contained 18 zygotes. At first, it seemed probable that the last nuclear division had been omitted in their case, and that they were simply zoospores of twice the normal size. But in three cases the nuclei were double structures—which, however, were not the daughter nuclei of a delayed final mitosis. They were, in fact, ordinary zoospore nuclei in the process of fusion. The sporangium was unusually large, and it may be that some accident prevented the cleavage walls from forming. That, however, does not alter the fact that intra-sporangial fusion of gametes took place in these few instances, an occurrence which serves to strengthen the belief in the normality of fusion between gametes of the same sorus. The wide question arises as to whether, in the particular case mentioned, the organism under the stimulating effect of the presence of toxic substances may not have had recourse to an earlier (? more primitive) method of sexual reproduction.

It has often been stated that, in nature, sori are developed in spring, while resting sporangia do not appear until late summer and autumn, by which time sori are no longer to be found, and it is hinted that the difference in the temperature of the seasons is responsible for the formation of one or other of the types of reproductive bodies. That more sori are formed at the beginning of the season and more resting sporangia at the end is certainly true, but resting sporangia may and do occur quite early, and sori similarly may be found quite late. Temperature is not the decisive factor, for the formation of resting sporangia may be induced at 18° C., while series of sori may be continued at 12° C. What is really essential for the fusion of gametes and the formation of resting sporangia appears to be that in two sporangia a maturation period should intervene between the formation of the zoospores and their discharge from the sporangium, and that the discharge from both

sporangia should be practically simultaneous. In spring and early summer, when there is an abundance of water in the soil, it is probable that one or other of the conditions will not be fulfilled, so zoospores remain unfused, and give rise to more sori. If, however, water is lacking at the critical period the zoospores have time to mature, and, the longer water is absent, the more sori will there be with their contents in the required condition. At the advent of water, therefore, numerous mature sporangia will be liberated together, and immediately discharge their zoospores (gametes). After the normal period of activity the latter will fuse, and resting sporangia will ensue.

The belief in the necessity of a maturation period intervening between gamete formation and discharge, before fusion can occur, is the outcome of an accident. A tumour bearing mature sori was left unwatered; some days afterwards, pieces of the surface of the tumour were placed in a drop of water and examined. The zoospores were seen to be extremely active, and, when their speed was reduced, to fuse in pairs. Water was then deliberately withheld from two other tumours bearing sori in a similar stage of development; on the fourth day the sori were examined in water, and in both cases the gametes fused. Sori and resting sporangia can be distinguished with a lens when in the living tumour, so the experiment was repeated on a larger scale without removing the sori, in order that the sporangia and gametes might remain under natural conditions. Tumours were chosen in which only maturing sori, and no resting sporangia, were present; they were well watered, and then left for four days, when they were again watered. In a few days, young resting sporangia were to be seen. On the other hand, similar tumours, which had been watered daily to serve as controls, still bore only sori. The experiment has been repeated many times, and in not one case have the matured gametes failed to fuse and give rise to resting sporangia, or the immature zoospores to give rise to sori. Moreover, the formation of sori, as of resting sporangia, has been induced at will over a wide range of temperature.

The Cytology of the Fusion Process.

When the gametes are fusing, they lie with their longer axes parallel or slightly inclined, and with their anterior ends facing usually in the same direction. In fig. 91*a*, two gametes may be seen approaching one another, while in fig. 91*b* they have touched. In fig. 92 they are pressed together, but the membrane of each is still distinct. The membranes, however, soon disappear at the surface of contact, and only a slightly denser strand of protoplasm then marks the line of union (fig. 93). The nuclei now approach each other, and eventually meet in the region of cell fusion. Stages in the approach of nuclei are shown in fig. 94 *a–c*. After meeting, the nuclei become pressed together, and the surfaces in contact are flattened (figs. 95*a*, *b*, *c*). The line of junction shows as a broad dark strand as the result of the accumulation of peripherally arranged chromatin of the two

gamete nuclei. The chromatin from the area of junction then moves to the poles of the nuclei, leaving the joined membranes clear (figs. 96*a*, *b*). The polar accumulations, however, soon disappear, the chromatin becoming distributed regularly on the outer surfaces of the nuclei (fig. 97). The polar depressions resulting from the junction of two oval bodies can no longer be detected, and the membrane between the nuclei ceases to be visible. This spherical fusion nucleus may contract slightly at its formation, and its chromatin forms a dense peripheral layer (fig. 98). This stage quickly passes, and the nucleus enlarges again, while the chromatin at the same time becomes resolved into granules (fig. 99), which, when the nucleus has reached its full size, are seen to be connected by an intricate network of linin threads (figs. 100, 101*a*, *b*).

Normally, the nucleus of the zygote is in this open, resting condition before infection occurs, the whole process of cell and nuclear fusion having been accomplished on or near the surface of the potato tissue. Certain abnormalities have been observed. They usually take the form of what is apparently the late arrival of a second zoospore after the first has begun to penetrate the epidermis. Provided penetration has not advanced too far, fusion is effected, and the zygote nucleus is formed. The fact that two zoospores have already fused does not act as a deterrent to a third, and cases have been found in which a zoospore is firmly perched on the top of an entering zygote.

No real membrane is formed round the zygote, but a slight condensation of protoplasm takes place at the surface, and, should plasmolysis occur, this outer layer remains in position, while the protoplasm within contracts (fig. 107).

Fig. 102 shows an aggregation of zygotes in a depression on the surface of a tumour. Two ruptured sori lie opposite the cavity at a little distance from it, but they are not shown in the figure. With the exception of a few zoospores which had settled in another depression they all entered the cavity depicted and there fused. No zoospores or zygotes were to be seen on the level portion of the tumour where cell division was not taking place rapidly, yet the cavity had a comparatively narrow opening and lay at a little distance from the sori. This attraction towards regions in which cell division is in progress may be observed all over the tumour.

Different stages of fusion are shown in the gametes and zygotes of fig. 103. In this case they have entered a channel between three rosette cells and there begun to fuse. On the lower cell there are four mature zygotes and several gametes, while above are to be seen different stages in the approach of the nuclei. The number of resting sporangia which occur close together in heavily infected tumours has led some to think that division of the organism after entry is necessary to explain the presence of so many. A glance at fig. 104, however, which is drawn from a section only 4 μ in thickness, shows how unnecessary is such an assumption, for which moreover, we have no evidence. Upon the large crumpled rosette cell at the upper right hand side there were 23 zygotes in addition to gametes. In living material on one occasion

35 zygotes, and on another occasion 26 were seen resting upon one rosette cell. These are admittedly exceptional numbers, but they occurred under natural conditions, and there is no reason to suppose that only a small proportion would have effected entry. In the section figured (fig. 104) one zygote is already completely within the host cell, while three are in the process of penetration, and several others are preparing to enter.

The Zygote.

The Entry of the Zygote.

The process of entry in all three types of penetrating bodies—soral zoospore, zygote, and zoospore from the resting sporangium—is essentially the same. The greater size of the last two bodies affords an opportunity for the observation of a more complete series of stages, but in the case of the zygote the advantage is outweighed by the possibility of confusion owing to the presence of two nuclei, two blepharoplasts and two strands connecting the blepharoplasts to the nuclei. As in the soral zoospore the cilia of the zygote are contracted before entry takes place. The process usually sets in early, before the fusion of the nuclei is completed. Owing to the difficulty in retaining cilia during manipulation it is unusual to find a zygote in which both are preserved, but in fig. 105*a* is depicted a zygote both of whose cilia are undergoing contraction. The process is not proceeding evenly. The shorter cilium is smooth in outline and comparatively broad, but in figs. 105*b*, *c*, two cases are shown in which the reduction has taken the form of a contraction in segments resulting in a series of small heavy joints. The latter type of contraction is the more general. In fig. 105*e* is a zygote just beginning to enter the host plant in which the relation of the various parts may be regarded as typical. The fusion nucleus has attained its final form, and consists of numerous peripheral chromatin granules and a central vacuole. The blepharoplasts and their connecting threads are distinct, and attached to one of the former is a contracting cilium. Between the blepharoplasts on the periphery lies the deeply staining tip of the nuclear projection. Subsequent stages in the contraction of the cilium are shown in figs. 105*f* and 105*g*, but of the fate of the knob after it reaches the surface of the zygote nothing could be discovered. When entry is in progress it is no longer to be seen although blepharoplasts are still visible.

The projection of the nucleus is formed without causing general derangement in its structure. In figs. 106*a*, *b*, *c*, are three stages in the development of the projection as it grows out from the nucleus towards the surface of the zygote in contact with the substratum. After the surface is reached the accumulation of chromatin in the apex of the projection increases in amount and eventually appears as a deeply staining granule, rounded towards the interior and flattened towards the surface of the zygote (fig. 107). One has the impression that the apical granule is being forced against the surface of the host cell which the organism is about to penetrate. This is an interesting example of the "control" by the nucleus of a special cell activity. The

apical granule of the nuclear projection now passes through the host cell wall and stages have been observed in which it is half inside the cell and half out (fig. 108). In fig. 109 the apical granule has passed completely through the wall. Owing to the obliquity of view the connection with the nucleus outside the cell cannot be completely demonstrated. Only nuclear substance can be recognised in the portion of the zygote which has entered and the amount of cytoplasm accompanying the granule seems to be extremely small. In fig. 110 is shown a slightly later stage in which a little protoplasm is already evident round the granule and that portion of the connecting strand which is inside the host cell. In fig. 111 the portion of the nucleus which has entered has begun to expand and has become irregular in shape; in this figure the blepharoplasts are also visible. When the nucleus before entry lies close to the lower surface of the zygote the length of the connecting thread is proportionally reduced, and in fig. 112 in which the nucleus is no doubt about to enter there is no projection, but the nucleus already lies in contact with the host cell. Further stages in the entry of the nucleus and cytoplasm are shown in figs. 113*a* and 113*b*; in the latter the nucleus is half way through the pore.

As the nucleus emerges into the host cell it expands into a dark structure of irregular shape which bears little resemblance to the original nucleus (figs, 114, *a*, *b*). Later, however, the nucleus becomes a more or less spherical body, but still remains homogeneously dark in staining reaction (fig. 115). Eventually the rest of the cytoplasm follows the nucleus through the pore (figs. 116, 117, 118), but as will be seen in fig. 119, the portion last entering appears as a small granule above the pore. After this has passed through the cell wall no trace of the pore can be observed. The nucleus for a short time remains drawn out towards the position of the pore after entry is completed. This extension is soon withdrawn however and the nucleus then becomes a dense homogeneous spherical body lying at the centre of the spherical mass of protoplasm. The parasite now moves down the host cell and begins what may for convenience be considered the first stage in the development of the resting sporangium.*

The Resting Sporangium.

The General Appearance of the Resting Sporangium.

Before entering upon a detailed account of the development of the zygote into the resting sporangium, a rapid glance will be cast at a few typical stages of the young sporangium in the tumour.

The general appearance of the epidermal layer of a tumour bearing resting sporangia in an advanced state of development is shown in fig. 120. The sporangia completely fill the host cells and bear upon their surface a series of ridges, the position

* Percival considers the stage after entry to be vegetative and the reproductive phase to start with the formation of a definite membrane other than the plasmatic one. As, however, the exact time of its appearance is very difficult to determine, it seems more convenient to make the above assumption.

of which corresponds with that of the intersection of the walls of the latter. No sori were present in the region of the tumour from which the section was cut, and the uniform size of the uninfected cells is typical of tumours which bear only maturing resting sporangia. The next six figures are drawn under the same magnification (400) in order to show the increase in size which the sporangium undergoes during its development. In fig. 121 the organism has passed down the host cell as far as the nucleus, but it is too minute to allow the detection of any structure, save the protoplasm and the nucleolus of the nucleus. It has enlarged and passed to the lower side of the host nucleus in fig. 122, and a few chromatin granules can now be seen lying beside the nucleolus in the clear vacuole. Division of the host cell has not yet taken place, but the young sporangium lies in a sufficiently deep position to ensure its inclusion in the lower cell should a transverse wall be formed. In fig. 123, on the other hand, division has taken place, and the sporangium now lies in a sub-epidermal cell. In the uniform mesh of its cytoplasm are several dark chromatin granules, while in the nuclear vacuole a small amount of linin has appeared. The host cell has become much enlarged, as the result of which the protoplasm now extends in long strands across the central vacuole to the parasite. In fig. 124 the deposition of the membrane of the sporangium has begun; the nucleus shows an increase both in its size and in the amount of linin it contains, while in the cytoplasm the vacuoles are becoming numerous. A second division of the host cell is responsible for the forcing of the sporangium of fig. 125 into the third layer of cells. In this example a little starch is present in the tissue of the tumour, but in the host cell the contents are reduced to a thin lining of protoplasm upon the walls and upon the sporangium. The granules in the cytoplasm have now become a conspicuous feature of the organism, while the membrane has developed almost to its full thickness. In the next figure an addition has been made to the membrane of the organism through the deposition of the dead contents of the host cell upon the outer sporangial membrane (fig. 126). In the mature sporangium the membrane thus consists of three layers, of which the two inner alone are of sporangial origin. The innermost, however, is too delicate to be visible under the magnification used. The nucleus in the figure lies in the midst of the enlarging granules of the cytoplasm, but its size is reduced, and under the pressure exerted by the expanding cytoplasm it has become shrunken to an irregular pointed structure. The nucleolus at this stage has given up most of its chromatin and appears pale and inconspicuous against the linin of the vacuole. The sporangium of this figure is at the most advanced stage which is normally to be found in a living tumour.

The Development of the Resting Sporangium.

At the beginning of this stage the nucleus of the parasite is represented as a single chromatic globule lying free in the mass of the cytoplasm (fig. 127). The first step in the development from this to a nucleus of normal type is the appearance of a

clear area round the globule (fig. 128). This area widens, and from the chromatic mass linin threads pass to its external limit, which about this time become sharply marked indicating the existence of a definite membrane (fig. 129). The origin of the clear area is obscure. Whether it arose as a vacuole in the cytoplasm, or was developed as the result of the pushing out of a membrane from the periphery of the chromatic granule, could not be determined.

The period during which the nucleus consists only of a large nucleolus and a clear vacuole is not of long duration, for there soon begins a process of transference of nucleolar substance to the nuclear vacuole similar in nature to that which takes place in the nucleus of the prosorus. As in the latter, the process leads to the depletion of the chromatic substance of the nucleolus, to the appearance of linin in the nuclear vacuole, and to the formation of chromatin granules in the cytoplasm. The transference takes place gradually by a succession of nucleolar discharges which may reach the number of six. This observation does not agree with those of Percival (35) and Bally (1), who describe only a single protracted discharge. The process is carried further in the resting sporangium than in the prosorus, for the nucleolus finally yields up all its chromatin and becomes reduced to an achromatic sphere in a shrunken and now probably functionless nucleus (fig. 126).

A stage immediately before the first discharge is shown in fig. 130. The nuclear cavity is now distinct and is traversed by one or two linin strands, while the large nucleolus stains deeply at the periphery. In the next stage small granules may be detected on a limited area of the surface of the nucleolus (fig. 131). They become pushed up above the surface by a slight enlargement of the region beneath them, and at the same time they increase in size until they constitute a compact mass of granules whose united volume occasionally is almost equal to that of the rest of the nucleolus (fig. 132). As the chromatin of the projection becomes dissolved a linin-like substance is revealed between the granules (fig. 133). During the process of the discharge the size of the nucleolus has greatly diminished, and its form may now be oval instead of spherical, but the intensity of its staining reaction at this stage is usually not reduced (figs. 134, 135). The granules of the nucleolar projection just described become completely dissolved and leave only the linin-like material in the vacuole (figs. 134, 135). This is the first addition of nucleolar linin to the vacuole. The irregular linin mass left after the dissolution of the chromatin has been termed by Percival (35) an "amœboid body." By the time the young sporangium has reached this stage of development, the host cell has probably undergone one division, and thus the first step in the deeper placing of the organism in the host tissue has been taken. Growth and development, however, continue uninterruptedly in the parasite during the host cell division. In the succeeding nucleolar discharges the amount of chromatin given off is greater than in the first, but in other respects they are similar, so a detailed description of the process will not be given in their case.

The large number of the chromatin granules lying in the cytoplasm, which are so characteristic a feature of the mature resting sporangium, appear usually after the second nucleolar discharge has taken place. These granules are formed at first in only a few strictly limited areas which lie symmetrically about the nucleus; fig. 136*a* shows four such areas. They are comparatively large, and their protoplasm stains more deeply than that elsewhere, while upon the strands which traverse them, minute granules can be detected. When the deposition of the granules has once begun, special staining reaction of the areas is no longer evident, but only the strands with their attached granules remain. Before this happens, however, new areas of deeper staining protoplasm appear between the original ones (fig. 136*b*). Several sets of such areas are formed, and the individual areas of each set are smaller than those of the preceding set. Moreover, as they appear and disappear rapidly only a short time elapses before the few large areas of the early series are replaced by numerous small ones of later formation. And these in their turn soon become inconspicuous among the great numbers of chromatin granules produced. But even in the case of granules formed late in the development of the sporangium a grouped arrangement can often be detected, although it may no longer be possible to distinguish any difference in the staining reaction of the protoplasm in their immediate vicinity.

Up to this point the parasite has shown only a plasmatic membrane, but, shortly after the appearance of the chromatin granules in the cytoplasm, the cell becomes bounded by a definite membrane, which at first stains deeply with gentian violet. Microchemical tests were not applied, but there can be no doubt that this new membrane is a definite cell wall.

In the nucleus of the sporangium of fig. 137, what is probably the third nucleolar discharge is beginning, and granules are now developing upon the surface of the nucleolus. A few chromatin granules have been formed in the cytoplasm, and the mesh of the latter, as is usual at this stage, is somewhat dense. In fig. 138 the membrane, although thin, is now quite distinct. In the interval which has elapsed between these two stages, the third discharge—as judged from the size of the organism—has been effected, the chromatin to a large extent has dissolved, and the nucleus has increased markedly in size. It is at about this stage that the nucleus attains its maximum size, when it may be as great as 22 μ in diameter, but, from now onwards, it will gradually decrease. A sporangium at a slightly later stage is shown in fig. 139. The solution of the chromatin in the nuclear vacuole in this sporangium has been completed, and an appreciable amount of linin has been added to the vacuole. The cell wall is becoming denser, and in the cytoplasm there is a marked increase in the number of granules. The sporangium of fig. 140, with its deeply-staining wall and numerous granules, is beginning to present the typical appearance of the sporangia of a tumour during the later half of the season. A slight decrease has already taken place in the size of the nucleus, and this reduction

will continue until it becomes an irregular, slightly staining, and no doubt functionless, structure, lying in the midst of the zoospore primordia. The structure of the sporangium at this stage presents sharp contrasts when stained with FLEMMING'S triple stain. The nucleolus is bright red, the chromatin both of the nuclear vacuole and of the cytoplasm violet, the cytoplasm itself orange, and the membrane deep violet. The staining reaction of the membrane, however, at this stage begins to change. From its first appearance, until it begins to thicken, it stains with chromatin stains, but as it becomes broader and its natural yellow colour is developed such staining is confined to its inner and outer surfaces, and eventually it stains only with such dyes as orange G.

The sporangia in figs. 141, 142, 143, show the early, mid, and late phases of what is probably the fourth nucleolar discharge. The membranes of the three sporangia show a great increase in thickness, and in the cytoplasm the granules are becoming both larger and more numerous as compared with fig. 140. The amount of protoplasm doubtless increases as the sporangia become older, but, during the expansion of the cell, the cytoplasmic vacuoles become so much larger that the protoplasm is reduced to fine strands. In the sporangium of fig. 143 there are two points of especial interest. The protoplasm of the host cell is more dense in the region which lies in contact with the sporangium; this is the first stage in the deposition of the cell contents which results in the formation of the outermost layer of the membrane of the mature sporangium. In addition, most of the granules in the cytoplasm now exhibit a differentiation of structure. The smaller still stain deeply and uniformly with chromatin stains, but in the larger the chromatin is confined to the periphery, while a lighter region becomes visible in the centre (fig. 143*a*). As enlargement of the granules continues, the lighter region is seen to be differentiated into plasmatic material and vacuoles. All the granules enlarge rapidly, and the smaller soon show the same differentiation as the larger (fig. 144). In this figure, it will be seen that several of the larger granules are multiplying by a process of constriction into unequal portions. This process is of frequent occurrence in sporangia of this age, but usually only a small number of the larger granules take part in it. In all the granules—which are actually the primordia of zoospores—a marked increase of chromatin now takes place (fig. 145), and it begins to accumulate at certain points, causing the primordia to become irregular in shape (fig. 146). As the accumulation continues, the chromatin projects from the surface, at first slightly and then more markedly. Finally, the chromatic projections become constricted and severed from the primordia, and they thus lie free in the cytoplasm of the sporangium. From one to five granules are given off at a time by each primordium, and the larger it is the more granules it emits. The process does not begin simultaneously in all the primordia, but eventually it occurs in all, even the smallest. At this stage the presence of the numerous minute granules in the cytoplasm between the primordia lends a peculiar appearance to the

sporangium (fig. 147*a*). The granules, however, soon cease to stain deeply, and usually they have almost disappeared when the primordia are still emitting occasional granules (fig. 147*b*). Soon after they have ceased to be emitted, the only remains of the granules are small, irregular, colourless structures upon the strands of protoplasm between the primordia (fig. 148). Since the longer zoospores emit more granules than the smaller, the inequality in their size is considerably reduced at the close of the process, and they become once more practically spherical. The chromatin is now reduced to a few small isolated granules, lying in the plasmatic material, composing the main mass of the zoospore body. The zoospores have been increasing in size for some time, and the protoplasm, which is now very dense, shows only small vacuoles. Eventually, the few small chromatin granules become replaced by a single larger one, though by what process is not clear (fig. 149).

Before the process of chromatin budding takes place in the young primordia, however, there are usually to be seen in each sporangium a number of exceptionally large primordia. These become constricted into two either during or just after the process of budding. In fig. 150*a* the difference in size of a normal and a large primordium may be seen, and stages of constriction are shown in figs. 150 (*b*–*f*).

The persistent chromatin granule increases in size, and becomes a conspicuous object in the otherwise lightly-staining zoospore. The cytoplasm meanwhile has become dense, and in many cases vacuoles are no longer discernible. As the zoospore approaches maturity, however, it apparently absorbs water, and a marked expansion takes place (fig. 151). Large vacuoles are now formed, and the cytoplasm becomes confined to strands, whose delicacy increases as the expansion continues; eventually, there is one large central vacuole, round which lie the slender strands of protoplasm. The single chromatin granule meanwhile remains near the periphery of the zoospore, and continues to enlarge. Before maturation, however, its homogeneous appearance is lost, and it becomes resolved into several small granules, while a clear area develops in their midst (fig. 152). In slightly older zoospores, a blepharoplast, with a thread connecting it to the nucleus, becomes visible, but its origin could not be determined (fig. 153). The blepharoplast, as in the soral zoospore, lies at the end of the body, opposite the nucleus, and the thread follows a curved path round the central vacuole. The zoospores are in this condition when they are discharged from the sporangium.

The Appearance of the Original Nucleus of the Resting Sporangium during the Later Stages of Development of the Zoospores.—After the sporangium has reached a stage similar to that shown in fig. 143 the chromatin in the vacuole as before, becomes completely dissolved. The size of the nucleus by this time is much reduced but its spherical form is still retained. On the disappearance of the chromatin of the vacuole the superficial portions of the linin matrix become diffuse and its substance is then gradually distributed in reticulate form throughout the vacuole. While this

distribution is in progress the matrix in many cases becomes separated from the nucleolus. As the linin reticulum is being formed the spaces in it are at first wide (fig. 154), but as distribution continues the width of the mesh is increased (fig. 155), and finally the vacuole is filled with a dense, homogeneous linin substance in which no reticulate structure can be detected. After each of the last few nucleolar discharges the nucleolus may remain in a vacuolated condition, but such vacuoles as are formed are never as large as those which arise after the discharges from the nucleolus of the prosoral nucleus. When the linin is uniformly distributed through the nuclear vacuole an occasional chromatin granule may be given off from the nucleolus, but the amount of chromatin in the nucleolus has been so reduced during the last few discharges that the nucleolus eventually remains as a scarcely perceptible, achromatic sphere usually lying excentrically in the nucleus.

The Formation of the Outermost Layer of the Wall of the Resting Sporangium.—When discussing the epispore of the resting sporangium JOHNSON (17) expressed the opinion that it is formed from "the residual contents of the host cell when not also from its cell wall as well." PERCIVAL (35) on the other hand thought its origin was to be referred to the cell walls alone, and this theory was supported by BALLY (1) who attributed its formation to the liquification of the original cellulose wall of the host cell.

No evidence could be found for BALLY'S suggestion that heavy liquefication of the host cell wall is mainly responsible for the formation of the membrane. On the contrary it is believed that it owes its origin to a change in the constitution of the contents of the host cell, and that it therefore does not consist of a single substance. The greater part of the membrane is formed from the sap contents of the host cell, but at the junction, with the true membrane of the sporangium at its inner surface and with the host cell walls at its outer, the host protoplasm also participates in its formation.

The stages in the deposition of the membrane can be best followed in material which has been fixed in an osmic acid solution and has not been bleached. In bleached material the cell sap in the early stages of membrane formation does not stain readily and as it is at that time naturally colourless, satisfactory stages cannot be obtained. In unbleached material, however, the peculiarly refractive appearance of the cell sap as its constitution begins to change serves as an indication of the progress of the deposition. The description of the process which will be given refers to unbleached material.

Before the outermost membrane is formed the sporangium usually occupies a more or less central position in the cell, and is surrounded by a single layer of large vacuoles separated by delicate layers of protoplasm. A thin layer of protoplasm immediately covers the surface of the sporangium and the walls of the host cell. As the protoplasm dies disintegration products of an oily nature are formed in the vacuoles, and these then appear as dark globules on the surface of the sporangium and of the host wall

(fig. 156). On the sporangium these globules are so numerous that they lie almost in contact with one another.

During the formation of these globules a change has taken place in the appearance of the wide portion of the cell occupied by the sap. The vacuoles are no longer clear but are filled with a substance—apparently gelatinous—which in fixed material has a peculiar milk-white sheen (fig. 157). The sap now forms a jelly and, in the process, sporangium, dead protoplasm, vacuolar region, and host cell wall are united into a firm whole. The milky appearance is now replaced by a clear translucence, and a faint yellow shade becomes discernible in the newly-formed membrane. The colour changes gradually to the deep orange characteristic of the outermost layer of the mature sporangial membrane, but the disintegration globules remain unchanged at its inner and outer surfaces (fig. 158). Owing to the presence of these globules the membrane of the sporangium when examined in surface view seems to be perforated by numberless holes. Sometimes the epispore is completely broken away in the subsequent development of the sporangium. Usually, however, it persists until the time of zoospore liberation, and it is possible that the spaces in the dead cytoplasm between the epispore and the true membrane originally occupied by the oily disintegration globules, are of service in retaining moisture.

The Division of the Infected Host Cell.—It is always actively dividing regions of the host plant which become infected, and as Bally (1) first stated it is owing to the repeated division of the infection cell that the sporangium becomes deeply placed in the tumour. As, however, he figures only isolated cases of host cell division, a more connected series of events will be described in the present instance.

The zygotes often congregate in such numbers that the majority of the epidermal cells of a given region become infected. Moreover, infection of the different cells is almost simultaneous. After infection has taken place it is usual to find that a large proportion of the epidermal cells divide within a short time of one another, and epidermal cell-division is thus normally widespread. As the result of this division most of the parasites lie in the inner (*i.e.*, lower) of the daughter cells. These infected sub-epidermal cells, however, do not divide so regularly, and in their case cells in which division is taking place are not often to be found near one another. As division is repeated and the sporangia become more deeply placed, the more rare becomes the simultaneous division of cells containing parasites.

In fig. 159 is shown a portion of the epidermis of the host tissue when the widespread first division is taking place. In two cells the new walls have been formed parallel to the surface of the tumour, and in a third the wall is set obliquely to the surface. Occasionally the wall is formed at right angles to the surface; when this occurs in cases of multiple infection the parasites are distributed laterally but not buried. In fig. 160 are four adjacent epidermal cells, each containing several parasites of different sizes, but division of the host cell has not yet taken place. Examples of the division of epidermal host cells in which the nuclei are in prophase, metaphase,

and telophase respectively, are shown in figs. 161, 162 and 163. Division of a sub-epidermal cell which was one of the daughter cells formed at the first division after infection may be seen in fig. 164. In this example the wall of the second division, as so frequently happens, will be set obliquely to that of the first division. Division (prophase) of a deeply placed host cell is shown in fig. 165.

Usually, only one maturing resting sporangium occurs in each host cell. Nevertheless, cases of multiple infection of epidermal cells are more common than cases of single infection. Under normal circumstances, division of the infected cell is repeated until the distribution of the parasites through the host tissue is effected. In figs. 166*a*, *b*, which are drawn from adjacent sections of one cell, six parasites can be seen, and, of these, three lie in the inner daughter cell and three remain in the outer. If parasites are left in the epidermal daughter cell, as in this case, or if it should be infected a second time, the cell will continue to divide until the parasites are removed to the tissue below. Thus the final deep position of the parasites is usually due to the repeated divisions of all the daughter cells.

Treatment of the Resting Sporangium and its Relation to Maturation.—The germination of the resting sporangium was secured in potato solutions by Johnson (17), and, although empty sporangial cases were observed in those in weak sugar solutions, it was believed that the zoospores were liberated only when the sporangia had developed in contact with, or in decoctions of, the host plant. Percival (35), however, found that material which had been saved from the previous autumn germinated in spring when suspended in drops of water for five or six days. In the absence of details of their treatment during the winter, however, it is presumed that the sporangia were retained in the decaying tumour, and evidence of the complete development of the sporangia in water was therefore lacking. In the course of the present investigation, it has been found, however, that sporangia removed early in the season from still healthy tissue, can mature and discharge their zoospores when kept in water alone, the length of the maturation period depending upon the state of development of the sporangia when removed. The period for those taken from living tumours soon after the formation of the outermost (sporangial) membrane, and therefore to be regarded as young, was $2\frac{1}{2}$ months. Whether the medium was rain water, soil water, or potato solution of not more than 1 per cent., made very little difference, as the longest and shortest times taken for discharge differed only by about ten days. Rain water, however, produced the quickest germination.

Ripe sporangia may be secured by the simple method of leaving them in the tumour and moistening them from time to time. But, when the progress of the sporangia is to be followed from day to day, isolation is necessary. It was found more convenient to work with large numbers and risk the loss of a certain percentage than to preserve a small number more carefully. Each batch accordingly contained thousands of isolated sporangia, and, although it might seem that great

labour was necessary to secure so many, they are so well protected by thick membranes that the labour of collection is comparatively slight. The tissue of the tumour was teased as finely as possible, and then moistened and rubbed lightly with the finger in a mortar, until the pieces were reduced to about the size of coarse sand grains. Sporangia separate from tissue more readily than cell from cell, and upon this fact the following rubbing and filtering processes depend. By filtering through muslin, the larger pieces of tissue were held back, and the filtrate, containing groups of sporangia, was poured into a funnel covered with ordinary silk. This was then spread on a flat dish, and the sporangia were again rubbed with the finger. By this means nearly all the sporangia were rendered free, and the mesh of the silk was sufficiently narrow to permit their passage on to the glass below, while the larger fragments of tissue were held back. Sediment of the same size as the sporangia was worked through with the latter, but most of it could be removed by centrifuging. After this drastic treatment, groups containing two or three sporangia might still remain, but fully 90 per cent. of the material consisted of isolated sporangia. No attempt was made to keep the sporangia sterile, as comparative freedom from bacteria was all that was required, and this was secured by repeated centrifuging in fresh solutions.

No difference was perceptible in the rates of development of cultures kept in diffuse light and in darkness, but strong illumination was avoided. Development continued between the temperatures of 9° C. and 18° C., the optimum lying between 12° C. and 14° C. In some cases the sporangia were immersed in the media, but usually they were kept only moistened, by being placed on germinating dishes standing in a little solution, or on boulting silk which was kept wetted with the media. The silk afforded a convenient means of noting rapidly the condition of the sporangia, for it could be spread upon a glass plate, and the sporangia inspected directly under the microscope.

From time to time sporangia were fixed, and large numbers were used. They were fixed under reduced pressure in the usual way, and washed in changes of water. A small portion was then cut from a test-tube about $\frac{3}{4}$ inch from the open end. A piece of boulting silk was drawn tightly across the rimmed end and secured by tying. It was moistened, and the sporangia were then gradually poured into it. When the water drained away, a thin layer of very weak agar solution, which had cooled as much as possible, was poured over the sporangia. Just as it solidified, the fastenings were cut, the glass removed, and the silk gently drawn from the agar. The sporangia were retained in the lower portion of the film, and the latter, after being cut into pieces of a suitable size, was dehydrated, imbedded, and sectioned in the usual way.

The Discharge of the Zoospores.

Shortly before the maturation of the zoospores the sporangium enlarges rapidly, and its diameter is often increased by as much as 50 per cent. The outer membranes are now quite soft and much distended, and the hyaline appearance of the innermost membrane becomes most striking (fig. 167). The last stage of development results in the wider spacing of the zoospores, during which processes the full length of the cilium is attained. As the zoospores mature, one portion of the innermost membrane of the sporangium swells up, forming a conical projection, which extends inwards, sometimes as far as the centre of the sporangium (fig. 168). The volume occupied by the zoospores is in consequence much reduced, and great pressure is exerted on the already softened outer membranes. Eventually the sporangium bursts, and the slit formed may extend almost round its periphery (fig. 169).

The zoospores are usually in active movement before the sporangium bursts, and this activity may continue for about two hours. When moving within the sporangium they are particularly sensitive to light. On one occasion, a sporangium containing active zoospores was kept in a weak, dark green light for an hour. By the end of that time the zoospores had gradually all come to rest. The brightness of the light was then increased. In five minutes some of the zoospores were moving; in ten minutes movement was general and rapid; and, shortly afterwards, the dashing to and fro of the earlier swarming period was resumed. When the zoospores are free from the sporangium, however, change in the brightness of the illumination has not been found to have such an appreciable effect upon their rate of movement. The activity of the zoospore of the resting sporangium is stated by Percival (35) to continue for twenty minutes. On several occasions, however, it was found that, provided the temperature did not rise above 15° C., movement continued for fully two hours, at the end of which time those zoospores that were in contact with the host plant entered the epidermis; those, however, which were not so situated, disintegrated. No zoospores were seen to become amœboid, but this condition of the zoospore has been so frequently reported that its occurrence cannot be doubted.

The Structure of the Zoospore.

The zoospore of the resting sporangium is about 2 μ in length and is slightly larger than that of the sorus, but the two closely resemble one another in appearance. The hyaline, main portion of the zoospore narrows at the refractive, anterior end where the nucleus is situated (fig. 171). The cilium is long and slender; the relatively short cilium of the figures of earlier workers is suggestive of immaturity of the zoospore. Several chromatin granules are to be found on the inner surface of the nuclear membrane and connecting them are a few slender strands of linin (fig. 172). The centre of the nucleus, as in the soral zoospore, is quite clear, and no definite nucleolus can be recognised.

The Entry of the Zoospore into the Host Tissue.

The zoospore usually comes to rest upon its side and, as in the soral zoospore, the nucleus, the blepharoplast and the thread connecting the two are all that comprise the nuclear apparatus before entry into the host plant begins (fig. 174). A slight protruberance, however, is soon noticeable on the side of the nucleus nearest the surface of the zoospore in contact with the host epidermis (figs. 175, 176). The projection extends further and further from the nucleus and it becomes correspondingly attenuated, until the final slender form is attained as it reaches the surface (fig. 177). When the nucleus lies close to the surface of contact the projection has not far to travel, and so is not so fine when it reaches its final position (figs. 178, *a–d*). The end of the projection eventually is pressed against the surface of the zoospore and it becomes slightly flattened (fig. 179). Entry into the host tissue then begins at the point of contact of the disc-shaped end of the projection with the host tissue. The cilium has meanwhile been withdrawn just as in the case of the soral zoospore (figs. 178*a*, 180). The granule-like apex of the nuclear projection is the first visible portion of the zoospore to enter, and in very early stages when its substance has not completely passed through the wall it appears as two small dots on either side of the pore (fig. 181). Protoplasm of the zoospore probably envelops the nuclear apparatus as entry is taking place but it cannot be detected in the early stages. In fig. 182, however, a small amount of cytoplasm has passed within the host cell, where it has already assumed a spherical form. In this example the host cell is plasmolysed, and the nucleus of the zoospore may be seen traversing the space between the wall and the protoplasm. Only a small portion of the zoospore remains upon the surface of the epidermis in the two examples of fig. 183, and in both cases the nucleus has almost completed its entry. The stage just after entry is shown in fig. 184; the main portion of the nucleus now occupies a central position in the organism but a portion still extends towards the pore and this will be drawn up before the spherical form characteristic of the transitional stage of the prosoral nucleus is acquired (fig. 185). Chromatin is formed rapidly and the nucleus soon becomes homogeneous, and the parasite in the meantime travels down the cell. A small clear area now forms round the nucleus, and as this vacuole widens, and a few linin strands appear across it, the stage is reached which was chosen as the starting point in the description of the life-cyle of the fungus.

General Discussion.

The Relation of Fungus and Host Plant.

If the fungus enters young host tissue the resulting tumour probably exhibits no trace of the form of the infected organ, although a slight conducting system is usually developed. When the fugus enters older tissue, however, the organ containing the

parasite may exhibit only local malformation, its tissues elsewhere being normally differentiated.

Although the tissue of the tumour differs in structure from that of uninfected regions of the plant yet it retains much of its normal power of reaction. Thus the cuticle on the surface of the tumour does not develop normally, yet when bacteria enter an epidermal cell of the tumour they are cut off by a cork cambium just as in other parts of the plant.

The behaviour of the infected cell varies according to whether it contains a prosorus or a resting sporangium, death being preceded in the one case by enlargement, and in the other by repeated division. When both zygote and zoospore enter one cell the effect due to the prosorus seems to predominate, for the cell enlarges more than usual and then dies without dividing. The more prosori the cell contains the greater is its enlargement.

The nuclei of injured cells of the tumour exhibit in especial degree the reaction to injury described by MIEHE (33)—that of migration into adjoining cells. If a superficial portion of the tumour is removed and left for twenty minutes before being fixed, it will be found that a general migration of nuclei has taken place in the cells adjoining those cut, the direction of movement being usually from the cut surface. On several occasions two nuclei have been observed entering one cell, and one nucleus leaving its cell as a second entered.

The Persistence of the Organism in the Soil from Year to Year.

Several instances have been reported of the infection of normally healthy potato "seed" in fields which were known to have borne previously a diseased potato crop, but in which no potatoes had been planted for eight years or longer. How the organism maintains itself in the absence of the potato plant is at present unknown.

COTTON (5) has succeeded in infecting other species of *Solanum* with wart disease, thus raising the question of the practical importance of the plurality of hosts. Although it is improbable that species of *Solanum* are present in the neighbourhood of the potato fields in sufficient numbers to be of economic importance in the maintenance of the parasite from season to season, yet certain species of *Synchytrium* are parasitic upon so many and such widely diverse host plants (*e.g.*, *S. aureum* reported to occur on 130 species), that it is possible that *S. endobioticum* also may be found in more plants than are at present recognised as its hosts, and that these may be of sufficiently common occurrence to serve as alternate hosts in the absence of the potato plant.

On the other hand it has been stated several times that the zoospore of the resting sporangium, after swimming freely, passes into an amœboid condition. This suggests that the organism may live for a long time sapropytically in the soil and possibly by repeated encystments may tide over many winter periods.

But there is a third possibility. Resting sporangia have been found to mature

and discharge their zoospores when in water alone. But probably in nature a small proportion of sporangia remain undischarged each year. It is doubtful, however, whether after eight years, sufficient would remain ungerminated to cause the wide-spread infection reported. In these cases the explanation of the "persistence" of the organism probably lies in the introduction, at planting of the second crop, of tubers which were apparently healthy but which really bore free resting sporangia upon their surface.

What is required, therefore, is to discover in the first place whether the organism really persists for a long period in the soil, and, if it should be proved that it does so exist, to discover whether the resting sporangium remains ungerminated, or whether the infection of other host plants, or the assumption of a saprophytic mode of life, is responsible for the maintenance of the fungus.

Immunity from Wart Disease.

It is a well known fact that certain varieties of potato are immune to wart disease, but the cause of this immunity is still to be discovered. In the case of the immunity of certain wheats to *Puccinia glumarum*, Miss MARRYAT (29) has shown that, "though the fungus succeeds in making good its entry and producing hyphæ, further progress is either completely checked by the breaking down and death of the host tissue locally, accompanied by the starvation and death of the parasite," or else, in cases of partial immunity, "a more protracted struggle takes place . . . and the development of the fungus proceeds to a farther point, but is still greatly retarded."

In the varieties of flax immune to *Fusarium Lini*, it was found by TISDALE and JONES (18) that in this case also the fungus succeeds in penetrating the host plant. In the susceptible varieties, the parasite penetrates directly to the vessels of the root. "In the resistant individuals, on the other hand, the invasion advances more slowly, and, before it reaches the vessels, is checked, and permanently walled off by the development of a corky layer."

So far, we have no indication wherein the immunity to wart disease lies. It may be due to a reaction between host and parasite similar to one or other of the above cases. On the other hand, it is possible that infection does not occur at all, possibly because the zoospores are not attracted to immune plants, or because a physical obstacle, such as a thick cuticle, intervenes. It is intended shortly to undertake an investigation of the mutual reaction between the parasite and immune varieties of potato.

Nuclear Reduction.

The fusion of the gametes is a sexual process involving a subsequent nuclear reduction. But, in the stage in which it is natural to expect the reduction to take place—the development of the resting sporangium—mitosis does not occur. A large amount of chromatin, however, is accumulated in the zoospore primordia of the resting sporangium, and the greater part of it is later thrown off, after which

process the typical zoospore nucleus is developed. The zoospore of the resting sporangium produces the sorus, but, in the development of that organ, we fail to find any nuclear division which suggests a reduction. A typical reduction thus appears to be completely absent from the life-cycle. It is therefore concluded that the rejection of chromatin by the primordia of the zoospores of the resting sporangia must be regarded as equivalent to the nuclear reduction of the higher plants, the haploid generation thus starting at the maturation of these zoospores.

The Relation of the Fungus to the Genus Pycnochytrium (DE BARY), SCHRÖT.

The diagnosis of the genus *Pycnochytrium* (DE BARY), given by SCHRÖTER (45) in 'Die Natürlichen Pflanzenfamilien,' reads as follows: "Fruchtkörper endogen, nach vollendeter Reife mit einer festen Haut umgeben. Schwärmsporangiensori nicht unmittelbar aus dem erwachsenen Fruchtkörper gebildet, sondern dadurch, dass sich der Inhalt des Fruchtkörpers durch eine feine Oeffnung entleert und eine dünnwandige Zelle bildet, deren Inhalt in Sporangien zerfällt. Einzelsporangien dicht gelagert, durch den gegenseitigen Druck vielkantig, oft unregelmässig gestaltet; Entleerung durch kurze, warzenartige Mündung; Membran farblos. Schwärmsporen rundlich, mit 1 Cilie. Dauersporangien aus einem erwachsenen Fruchtkörper gebildet; dick Membran, braun." From the account which has just been given of the development of the fungus at present under consideration, it will be seen that it exhibits all the characteristics enumerated by SCHRÖTER in the diagnosis of the genus *Pycnochytrium* (DE BARY), SCHRÖT.

This genus is divided into three sections, the description of one of which reads: "Die Entleerung des ursprünglichen Fruchtkörpers und Ausbildung der Schwärmsporangien erfolgt schon auf der lebenden Nährpfl. in der ursprünglichen Nährzelle. Nebenher werden noch Dauersporangien gebildet, welche eine längere Ruhepause durchmachen."

In this division—called by SCHRÖTER *Mesochytrium*—the present fungus may be included, for in all four points its development follows the description given. In the three species of this section, the contents of the prosorus migrate either in the upward or in the downward direction in the host cell; *Pycnochytrium Succisæ* (DE BARY and WORONIN), SCHRÖT., is the only species in which the migration is upwards. The number of sporangia formed in the sorus is greater in *P. Succisæ* than in *Synchytrium endobioticum;* wide variation in this respect, however, may be seen throughout the genus. The only other major distinction between the two species is that of the colour of the oil in the cytoplasmic vacuoles, that of *Pycnochytrium Succisæ* being orange and of *Synchytrium endobioticum* hyaline. The other sections of the genus *Pycnochytrium*, however, exhibit the same colour distinction, and its extension to the section *Mesochytrium* was to be expected as our knowledge of the genus widened.

Since, as has been shown above, the present species exhibits without exception all

the characteristics of the genus *Pycnochytrium*, the retention of the name *Chrysophlyctis endobiotica* (SCHILB.) is no longer justified. Whether the organism is called by the broad generic name *Synchytrium*, as suggested by PERCIVAL (35), or by SCHRÖTER's narrower generic term *Pycnochytrium*, depends on whether FISCHER's or SCHRÖTER's classification of the *Synchytriaceæ* is accepted. As the reasons with which SCHRÖTER justifies his resolution of the old genus *Synchytrium* into two new genera—one of which, though narrower in scope than the original genus, retains the name *Synchytrium*, while the other is named *Pycnochytrium*—do not seem to be sufficiently valid to warrant the change, the writer prefers to recognise FISCHER's classification of the wider genus *Synchytrium*. In consequence, as will be seen from the title of the present paper, it is not suggested that the generic name *Synchytrium*, given to the organism by PERCIVAL (35), should be changed to that of *Pycnochytrium* (DE BARY), SCHRÖT.

Sexuality in the Synchytriaceæ.

Although the occurrence of sexuality in the *Synchytriaceæ* has long been suspected, this is the first case in which the actual process of fusion has been observed. But the accounts of early workers (DE BARY (2), WORONIN (50), SCHRÖTER (44)) contain so many references to "giant zoospores" possessed of two cilia—which were probably zygotes—that there is a great possibility that sexual fusion similar to that of the present species will be found to take place in all those members of the (older) genus *Synchytrium*, in which true resting sporangia are formed.

SUMMARY.

The occurrence of sexuality in the *Synchytriaceæ* has long been suspected, but the fusion of motile isogametes recorded in this paper is the first instance in which a sexual process has been observed.

Two forms of reproductive bodies are formed in *Synchytrium endobioticum*, the resting sporangium, and the sorus. The resting sporangium produces zoospores. The sorus produces motile cells, which can behave either as zoospores or as gametes; there is evidence that treatment during the later stage of maturation of the sorus is of influence in determining the sexual or asexual nature of its motile cells. It would seem, however, that the gametes produced as the result of these special conditions during maturation are of facultative nature. It would seem probable also that the gametes fuse only with those of another sporangium.

The zoospores of the resting sporangium are almost oval in form, have a single long posterior cilium, and a bright anterior spot where the nucleus lies. The cilium is attached to a blepharoplast, and a fine chromatic thread joins blepharoplast and nucleus. When infecting the potato plant, the zoospore passes through the outer wall of an epidermal cell of young tissue. Nucleus and cytoplasm are much compressed during entry, but both resume a spherical form as the organism emerges

into the host cell. The body of the organism, from the time of entry to that of the cleavage into sporangia, has, for convenience, been termed the prosorus.

The young prosorus passes to the lower end of the host cell. The cytoplasm shows a fine network and the nucleus appears as a single homogeneous granule. From this granule a nucleus of more typical form arises by the development round it of a clear area across which run linin strands from granule to membrane. The granule may now be spoken of as the nucleolus. Whether the cavity of the nucleus so produced is of nuclear or of cytoplasmic origin could not be determined.

A process of nucleolar discharge sets in; globules, consisting of both chromatin and material, are formed on the nucleolus and their substance is given off into the nuclear cavity; the chromatin is dissolved, while the linin becomes distributed through the nuclear cavity. Three discharges take place at this phase; after the two earlier ones the nucleolus may again become homogeneous; at the close of the second discharge the nucleolus is found in contact with the nuclear membrane.

Prophase of the primary mitosis is considered to begin when the third nucleolar discharge sets in; at the close of this discharge the nuclear cavity is filled with a homogeneous mass of linin. At this stage fine strands, five in number, are to be seen extending from the nucleolus towards the membrane on the opposite side.

The host cell has enlarged and is now dead, while the parasite fills its lower half; the prosorus develops a thick orange outer, and a thin hyaline inner, membrane; great numbers of chromatic granules are present in its cytoplasm. The cytoplasm and nucleus of the prosorus, while surrounded with a delicate membrane, now pass through a pore in the upper region of the outer membrane into the host cell.

Repeated mitosis of the nucleus takes place before the cytoplasm has wholly emerged from the pore. There are five minute spherical chromosomes; it is believed that they originate from a globule given off by the nucleolus. The spindle is intranuclear in origin, the nuclear membrane persists until metaphase. No trace of asters or of centrosomes was found.

After about five nuclear divisions the prosorus segments into from four to nine sporangia, the walls being formed simultaneously and independently of the nuclei. The sporangia remain thin-walled and enclosed in the delicate common membrane. Nuclear divisions continue until 200–300 nuclei are formed in each sporangium. The chromatic granules in the cytoplasm have by now disappeared.

On the absorption of water a small vacuolated area appears round each nucleus, and separating the areas are dense intersecting strands of protoplasm. A chromatic granule, the blepharoplast, lies near the periphery of each area and is connected to the nucleus by a deeply staining thread. Separation of these areas is effected by the formation of the vacuoles in the middle of the dense delimiting strands. The parts so separated, which first appeared as the lighter areas, become the young zoospores. As separation continues the connection between the zoospores is reduced

to a single long strand running from the blepharoplast of one zoospore to the surface of another. Finally the strand becomes free at the end which is not attached to the blepharoplast and thus the cilium is produced.

Repeated division of the host tissue has meanwhile resulted in the formation of a tumour; in addition the epidermal cells in contact with the infected cell have grown outwards into a rosette of elongated refractive cells surrounding the infected cell.

At the time of the cleavage of the prosorus into sporangia the cells lying beneath the parasitised cell divide several times by walls lying almost parallel to the surface of the tumour. At the maturation of the sporangia these cells absorb water and enlarge, exerting pressure upon and raising the infected cell and its rosette above the surface of the tumour; pressure is exerted on the infected cell from within owing to the enlarging sporangia. Under the combined pressures the cell and soral membrane burst and the sporangia become free.

One or two hyaline projections are formed on the surface of the sporangium and through the rupture of one of these the motile cells are liberated. If the motile cell is a zoospore it develops into a sorus; if it is a gamete, it fuses with a similar gamete, probably from another sporangium, and the resulting zygote develops into a resting sporangium. The motile cells from the sorus are smaller than the zoospores of the resting sporangium, but are similar to them in structure.

The gamete nuclei fuse and the cilia shorten before the passage of the zygote into the host takes place. A nuclear projection plays a part in the process of entry.

The zygote after entering passes to the lower end of the host cell. The parasite nucleus appears as a spherical granule of chromatic material round which a clear space develops. The substance of the nucleolus (*i.e.*, the deeply staining granule) is then repeatedly given off into the nuclear vacuole; the chromatin of the discharged substance dissolves, the linin remaining in the vacuole. Soon after the nucleolar discharges begin chromatic "granules" appear in the cytoplasm of the parasite, and at the same time a cell wall is formed round the body of the young resting sporangium. The "granules" enlarge and show a differentiation into chromatic and achromatic material; these "granules" are the primordia of zoospores. The chromatic material, which is arranged in irregular strands near the periphery of the primordia, begins to project from the surface at several points, and a large proportion of it is then given off into the cytoplasm of the sporangium. Eventually a single chromatin granule is left in each young zoospore; from this granule the nucleus of the mature zoospore arises. A blepharosplast, with a deeply staining strand connecting it to the nucleus, is now formed.

The sporangial membrane shows three layers. The two inner layers of the membrane are derived solely from the parasite; the outer layer is formed from material derived from the disorganising host cell. The rupture of the resting sporangium results from the swelling of the innermost hyaline layer of the membrane.

In the accounts of early workers upon the *Synchytriaceæ*, and particularly in those

of De Bary (2), Woronin (50), and Schröter (44), so many references are made to *giant zoospores possessed of two cilia* that there seems to be a great probability of the occurrence of a sexual fusion, similar to that of the present species, in all those members in which true resting sporangia are formed.

The present species is shown to exhibit all the characteristics of a true *Synchytrium*, being closely allied to *S. succisæ*, from which it differs in the colour of the contents of the sorus and of the resting sporangium, and in the number of sporangia formed in the sorus. As Schröter's distinction between *Pycnochytrium* and *Synchytrium* does not appear to be a satisfactory one it seems preferable to include all the species of these two genera in Fischer's original genus *Synchytrium*, and the name *Synchytrium endobioticum* is accordingly retained.

In conclusion, the writer would express her indebtedness to Prof. V. H. Blackman, at whose suggestion the present investigation was undertaken, for his most helpful criticism throughout the course of the work.

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DESCRIPTION OF PLATES.

Plate 12.

Sorus: General.

Fig. 2.*—Young tumours bearing sori only; length of shoot, 7 mm.

Fig. 3.—Surface view of group of immature sori; a single depressed host cell in the centre of each rosette. Drawn from a photograph. × about 150.

Fig. 4.—Rosette containing enlarging host cell; rosette cells not yet divided. × 170.

Fig. 5.—Rosette cells divided; the prosoral contents have migrated obliquely; the outline of the host cell is indicated by a dotted line. × 170.

Fig. 6.—Side view of young rosette; host cell indicated by dotted lines. × 170.

Fig. 7.—Surface view of mature sorus showing three sporangia in a much enlarged host cell. × 170.

Fig. 8.—Surface view of ruptured host cell; three young prosori lie on the left of the rosette and three young resting sporangia on the right. × 170.

Prosorus.

Fig. 9.—Very young prosorus passing down towards the nucleus of the host cell; the cytoplasm of the prosorus is arranged in radiating strands, and the nucleus is still slightly irregular in shape. × 1650.

Fig. 10*a*.—Enlarging prosorus. The nucleus is still irregular in shape while the cytoplasm shows a reticulate arrangement. × 2000.

Fig. 10*b*.—The nucleus has much enlarged, but traces of the granules of the zoospore nucleus can still be seen. × 2000.

Fig. 10*c*.—The nucleus is spherical and homogeneously dark and lies free in the cytoplasm. × 2000.

Fig. 10*d*.—A clear area has arisen round the homogeneous granule. × 2000.

Fig. 11.—Young prosorus in an enlarged host cell. The contents of the host cell are much vacuolated. The nucleus of the prosorus consists of a central nucleolus and a large nuclear cavity containing chromatin which has arisen from a previous nucleolar discharge. The enlarged host nucleus rests upon the prosorus. × 400.

Fig. 12.—Young prosorus with minute chromatin granules in its cytoplasm, and a large nucleus in the cavity of which chromatin is undergoing solution. Disorganisation of the host-nucleus is beginning. On the right-hand side, the cell adjoining the host cell has grown up to form the rosette. A rosette cell is absent from the left-hand side as there another host cell adjoins. × 400.

* For fig. 1 see text-fig. on p. 412.

Fig. 13.—Prosorus shortly before the migration of its contents. It has a firm membrane and numerous chromatin granules in the cytoplasm. The nucleolus lies upon the nuclear membrane, and extending from it across the now densely filled nuclear cavity are several long linin threads. Lying immediately beneath the membrane in the upper part of the prosorus is the dense mass of cytoplasm which will begin the process of migration, and above which the pore will be formed. × 400.

Prosorus: Second Nucleolar Discharge.

Fig. 14.—Nucleus before discharge has begun. There is a large nuclear cavity, suspended at the centre of which, by a few linin strands, is the nucleolus. No chromatin is present in the nuclear cavity. × 800.

Fig. 14*a*.—Nucleus after first discharge; the nucleolus is vacuolated; linin and chromatin are present in the nuclear cavity. × 800.

Fig. 15.—Beginning of the second nucleolar discharge. Globules of chromatin are forming on the surface of the nucleolus. × 800.

Fig. 16.—The substance of the nucleolar globules has become opened up into an irregular interwoven mass of chromatin extending across the nuclear cavity. Occasional globules, which will be given off independently of a general discharge, are already to be seen upon the nucleolus. × 800.

Fig. 17.—The chromatin discharged from the nucleolus has undergone partial solution and now occurs as isolated granules, while the linin is becoming evident. × 800.

Fig. 18*a*.—The substance of the second discharge has been given off in a single globule, and a large vacuole has consequently been formed in the nucleolus. No chromatin remains in the nuclear cavity. The linin can be traced to the mouth of the nucleolar vacuole. × 800.

Fig. 18*b*.—The linin is becoming evenly distributed. The nucleolus contains several vacuoles. × 800.

The Third Nucleolar Discharge.

Fig. 19.—The globules of the third discharge are forming. The linin is now more or less evenly distributed, and the nucleolus is once more homogeneous in appearance. × 800.

Fig. 20.—A lightly staining, finely vacuolated nucleolus bearing the numerous globules characteristic of the third discharge. The nucleus has been relatively inactive and the chromatin in the cavity is not completely dissolved. The nucleolus now rests on the membrane. × 800.

Fig. 21.—The chromatin is being transferred from the globules to the long heavy strands. The linin arising from the previous discharges is uniformly distributed and almost homogeneous, only a slight trace of the earlier reticulum remaining. × 800.

Fig. 21*a*.—An early stage of transference of chromatin from globules to strands. × 1650.

Fig. 22.—Transference of the chromatin of a globule to strands; chromatin solution has begun. × 1650.

Fig. 23.—Chromatin solution advanced; granules isolated on the several distinct linin strands; the nucleolus is much vacuolated. × 800.

Fig. 24.—All the chromatin has disappeared from the strands which are now five in number and delicate in structure. × 800.

Fig. 24*a*.—A nucleus in which the chromatin has become separate from the strands and now lies as granules near the membrane. The nucleolus in this example has contracted before migration has taken place. × 800.

Fig. 25.—A nucleus in which the third nucleolar discharge has followed quickly upon the second; the linin in the cavity, due to the second discharge, has not yet lost its reticulate arrangement. × 800.

Fig. 26*a*.—Formation of the globules for a discharge in a young homogeneous nucleolus; their substance is drawn from regions near the surface, and the vacuole formed is not much larger than the globule. × 1650.

Fig. 26*b*.—Formation of globules in a vacuolated nucleolus. The globules are formed deep within the nucleolus and are much smaller than the vacuolar region from which they have drawn their substance. × 1650.

The Passage of the Prosoral Contents into the Host Cell.

Fig. 27.—Prosorus before the passage of the contents begins. The protoplasm beneath that portion of the membrane where the pore will be formed has become dense and free from chromatin granules. A slight cap of dense protoplasm surmounts the nucleus. × 800.

Fig. 28*a*.—The beginning of the passage. The dense portion of protoplasm alone has passed out; the edge of the outer membrane at the pore is turned up; the small portion of the inner lining is expanding to keep pace with the outflowing protoplasm which it will continue to cover; a slight deposition of the dead host cell contents can be seen on the outer membrane. × 800.

Fig. 28*b*.—The pore at a later stage; the edge is recurved; the heavy host cell deposit on the membrane is shaded, the outer membrane itself is represented as a clear envelope; the inner membrane can be traced from outside through the pore. × 1650.

Fig. 29.—Two prosori in a much enlarged host cell discharging at the same time, obliquely, and in opposite directions. × 400.

Fig. 30.—The nucleus has begun to move and is preceded by a dense strand of protoplasm originating from the cap which previously surmounted it; the dense protoplasm which lay beneath the pore is now spread out round the periphery of the expanding protrusion. × 400.

Fig. 31.—The apex of the nucleus has reached the pore, but the lower end has not yet left its original position. × 400.

Fig. 32.—The apex of the nucleus is through the pore and is already expanding as it continues to move forward; vacuoles have been formed round the periphery of the protoplasm within the original membrane. × 400.

Fig. 33.—The nucleus is more than half through the pore (slightly plasmolysed). × 400.

PLATE 13.

Fig. 34.—The nucleus reconstituted in its final position; the fine linin strands are evident and the nucleolus is budding. The vacuoles round the cytoplasm in the original envelope are now more conspicuous; the section is tangential to the host cell. × 400.

Fig. 35.—Two nuclear divisions have occurred, yet a few strands of protoplasm still remain in the original envelope; a vacuole has expanded into the region of the discharged protoplasm. The section is slightly tangential to the host cell. × 400.

Fig. 36.—Stages in the formation of the vacuoles at the periphery of the protoplasm in the original envelope :—

(*a*) Early; longitudinal section through the pore. × 400.
(*b*) Late; longitudinal section through the pore. × 400.
(*c*) Fairly late; section transverse to axis which passes through the pore. × 400.

Mitosis of the Primary Nucleus. Late Prophase.

(Prophase begins with the third nucleolar discharge before the passage of the prosoral contents.)

Fig. 37.—The five linin threads are loosely coiled together near the nucleolus; extremely delicate strands connect them to the nuclear membrane; the vacuolar region is filled with the homogeneous linin from the nucleolar discharges. × 1650.

Fig. 38.—The linin strands beginning to straighten. × 1650.

Fig. 39.—The linin strands are beginning to assume parallel positions across the centre of the nucleus; the nucleolus which is budding will pass out to one side. × 1650.

Fig. 40.—Three strands almost parallel; the nucleolus has passed to one side and bears a large globule, possibly for the formation of the chromosomes. × 1650.

Metaphase.

Fig. 41.—The spindle is long and slender, the chromosomes are five in number, spherical and minute; the nucleolus and two small chromatin granules are visible; the nuclear membrane still exists. × 1650.

Mitosis of the Secondary Nuclei of Prosorus and Sorus. Resting Stage.

Fig. 42.—Five nuclei of different sizes in the resting condition; the excentric nucleolus is evident only in the larger nuclei; the clear central cavity is traversed by several linin strands. × 1650.

Prophase.

Fig. 43.—The peripheral chromatin granules have almost disappeared, and a linin reticulum is formed across the nuclear cavity. × 1650.

Fig. 44.—The nucleolus is budding freely, the granules will, however, disappear later. Linin strands of the future spindle are evident and the nuclear cavity is homogeneous. × 1650.

Fig. 45.—Slightly later stage than fig. 44; the granules have disappeared; linin strands now distinct. × 1650.

Figs. 46*a*, *b*, *c*.—Three stages in the giving off of the large globule from which the chromosomes are probably formed. × 1650.

Fig. 47.—The five chromosomes; the spindle threads not evident. × 1650.

Metaphase.

Fig. 48.—The spindle is formed, and three of the five chromosomes are shown; the nuclear membrane still persists; the nuclear cavity is filled with homogeneous linin; the nucleolus lies to the side of the spindle. × 1650.

Figs. 49*a*, *b*.—The five chromosomes are evident. × 1650.

Anaphase.

Fig. 50.—Early anaphase; the daughter chromosomes have just separated; elongation of the spindle has begun. × 1650.

Figs. 51*a* and *b*.—Two stages in the separation of the daughter chromosomes; the portion of the spindle between the groups is maintained at its full width until late. × 1650.

Telophase.

Fig. 52*a*.—The elongated spindles have narrowed to a darkly staining thread; the chromosomes are uniting to form a chromatic granule. × 1650.

Fig. 52*b*.—The end of the spindle seems to be included in the chromatic granule formed from the uniting daughter chromosomes; the vacuole has been formed round the granule but is still narrow. × 1650.

Fig. 53.—The vacuole has widened, the granule is homogeneous, and a few linin strands have been formed; the remains of the spindle are still evident. × 1650.

Fig. 54.—The evolution of the resting nucleus; granules are being given off from the nucleolus in fig. 54*b*. × 1650.

Fig. 55.—As fig. 54, but the nuclei are smaller. In figs. *b* and *c* globules are being given off from the nucleolus in the formation of peripheral granules of the nucleus. × 1650.

Segmentation of the Prosorus.

Fig. 56.—Early stage in segmentation; the protoplasm is denser along the course of the future walls. × 800.

Fig. 57.—An undulating, deeply staining layer of protoplasm is visible, down the middle of which the membrane will be formed. × 2300.

Fig. 58.—The undulating layer has become straighter and in the middle a delicate membrane is differentiated. × 2300.

Fig. 59.—The newly formed membranes intersect one another at definite angles. × 2300.

The Sorus.

Fig. 60.—A sorus showing five sporangia shortly after the formation of the cleavage membranes; the nuclei already show a difference of one generation. The section is tangential to the host cell; slightly plasmolysed. × 400.

Fig. 61.—Longitudinal section of host cell showing two sporangia of a sorus above, and an empty prosoral membrane below; the nuclei have undergone several divisions. × 400.

Fig. 61*a*.—The nuclei have become further reduced in size, and are all of the same size. × 400.

Fig. 62.—A portion of a sporangium shortly before the delimitation of the zoospores. The nuclei have a clear central cavity and one or two peripheral chromatin granules, but a nucleolus cannot be distinguished. The cytoplasm is now dense and no chromatic granules can be seen in it. × 1650.

The Formation of the Zoospores.

Fig. 63.—The zoospore areas are delimited and some are becoming separated through the formation of vacuoles down the middle of the delimiting strands. The nucleus, blepharoplast, and deeply staining strand connecting the two can be seen. × 2300.

Fig. 64.—The separation is more advanced than in fig. 63. × 2300.

Fig. 65.—The strands which persist are now unequal in length; the zoospores are enlarging. × 2300.

Fig. 66.—The strands are joined at one end to a blepharoplast and at the other (upper) end to the body of a zoospore (but not to its blepharoplast) or to similar long threads. On separating the threads become the cilia of the zoospores. × 2300.

The Liberation of the Sporangia.

Fig. 67.—The cells beneath the host cell have enlarged and exerted pressure upon the host cell from below. × 170.

The Formation of the Rosette.

Fig. 68.—The prosorus has already reached a moderate size but no elongation has yet taken place in the epidermal cells immediately adjoining the host cell. × 170.

Fig. 69.—The prosorus has begun to discharge and the adjoining cells have become elongated. × 170.

Fig. 70.—The prosorus has completed its passage and the epidermal cells have grown up with the enlarging host cell, leaving however a small uncovered area on the surface of the host cell. × 170.

Fig. 71.—The cells of the rosette after the liberation of the sporangia. A portion of the original envelope of the prosorus is still attached to the host cell wall. × 170.

The Discharge and Structure of the Zoospores.

Fig. 72.—A sporangium swelling prior to the discharge of the zoospores; a hyaline projection is formed. × 780.

Fig. 72*a*.—Three mature sporangia at the base of a host cell; zoospores are already liberated in the cell; the common soral envelope surrounds the sporangia; a small portion, in section, of the original envelope of the prosorus is to be seen, against one wall of the cell. × 400.

PLATE 14.

Fig. 73.—Living zoospores; the refractive anterior spot denotes the position of the nucleus. × 780.

Fig. 74*a*.—Zoospore showing nucleus, blepharoplast, and the strand connecting it to the nucleus; the nucleus has a clear central cavity and a few peripheral chromatin granules, but no distinct nucleolus. × 1650.

Fig. 74*b*.—Fixed zoospore, but unbleached and stained with HEIDENHAIN'S iron-alum-hæmatoxylin, showing nucleus, blepharoplast, and fat globules adjoining the nucleus. × 1650.

Entry of the Soral Zoospore.

Figs. 75*a–f*.—Stages in the retraction of the cilium. × 2000.

Fig. 76*a*.—Zoospore on the surface of the host plant; the projection just leaving the nucleus. × 2000.

Fig. 76*b*.—The projection has advanced almost to the surface of the zoospore in contact with the host cell. × 2000.

Fig. 77.—The projection from the nucleus has reached the surface of the cell, and the portion between the apical granule and the nucleus now appears as a deeply staining thread; the blepharoplast is also visible. × 2000.

Fig. 78.—The earliest stage of entry; the apical granule of the nuclear projection is half way through the host wall; surface view. × 2000.

Fig. 79.—The apical granule of nuclear projection is within the host wall, and the strand connecting it to the nucleus without can be seen. × 2000.

Fig. 80.—Stage of entry slightly later than fig. 79. × 2000.

Fig. 81.—Surface view of entering zoospore; a small portion of the cytoplasm has entered in addition to the apical portion of the nuclear projection. × 2000.

Fig. 82.—The main body of the nucleus is elongating towards the entry pore. × 2000.

Fig. 83.—The nucleus has almost completed its entry. × 2000.

Figs. 84 and 85.—The nucleus is wholly within the host wall and only a vesicle of cytoplasm remains without. × 2000.

Fig. 86.—Both nucleus and cytoplasm have completely entered, but the nucleus still remains connected to the pore, although its main part has already resumed its spherical form. × 2000.

Fig. 87.—The zoospore (prosorus) beginning to travel down the host cell close to a side wall. × 2000.

Fig. 88.—As fig. 87; slightly older prosorus with reticulately arranged cytoplasm. × 2000.

Fig. 89.—The nucleus of the prosorus is now enlarged and homogeneous and a clear area is appearing round it. × 400 and 2000.

Fig. 90.—The prosorus further enlarged; the typical nuclear cavity is now formed, and the chromatic globule is suspended in the centre. × 2000.

The Fusion Process.

Fig. 91*a*.—Two gametes approaching each other. × 2000.

Fig. 91*b*.—The gametes have touched, but face in slightly different directions in the two examples. × 2000.

Fig. 92.—The gametes are pressed together, but the membranes are still distinct; unbleached, stained with HEIDENHAIN'S iron-alum-hæmatoxylin. × 1650.

Fig. 93.—The membranes have disappeared from the region of contact and only a slightly denser strand of protoplasm marks the line of union. × 2000.

Figs. 94*a–e*.—Stages in the approach of the gamete nuclei. × 2000.

Figs. 95*a*, *b*, *c*.—The nuclei become pressed together and the surfaces in contact flatten. × 2000.

Figs. 96*a*, *b*.—Stages in the movement of the chromatin from the joined membranes to the poles of the nuclei. × 2000.

Fig. 97.—The polar accumulation of chromatin becoming distributed round the outer surfaces of the nuclei; the joined membranes are now free from chromatin. × 2000.

Fig. 98.—The fusion nucleus; the chromatin is arranged round the periphery in a broad irregular strand. × 2000.

Fig. 99.—The chromatin of the fusion nucleus is becoming resolved into granules. × 2000.

Fig. 100.—The fusion nucleus now has isolated peripheral chromatin granules and a single strand of linin. × 2000.

Figs. 101*a*, *b*.—The mature zygote nucleus; a more intricate system of linin threads has now developed. × 2000.

Fig. 102.—An aggregation of zygotes in a depression on the surface of the tumour; two ruptured sori lie near but are not shown in the figure. The figure is drawn from a section. There were as many as 184 zygotes in this depression. × 780.

Fig. 103.—Gametes and zygotes in a channel between three rosette cells; on the lower cell there are four mature zygotes and several gametes; above are different stages in the approach of the zygote nuclei. × 1650.

Fig. 104.—From a section 4μ in thickness, showing in what large numbers the zygotes cluster on the surface; the large cell to the right-hand side is a crumpled rosette cell. × 800.

The Entry of the Zygote.

Figs. 105*a*–*g*.—Stages of contraction of the zygote cilia; the nuclei are at different stages of fusion; in fig. *e* the zygote is already beginning to enter. × 2000.

Figs. 106*a*, *b*, *c*.—Three stages in the development of the nuclear projection; in two of the zygotes blepharoplasts are visible. × 2000.

Fig. 107.—The nuclear projection has reached the surface of the zygote; chromatin is accumulating in the apical granule; plasmolysis has occurred. × 2000.

PLATE 15.

Fig. 108.—Surface view of early stage of zygote entry; the apical granule of the nuclear projection is half way through the host wall. × 2000.

Fig. 109.—Three stages of early entry; the apical granule has wholly passed through the wall; in two cases blepharoplasts are visible. × 2000.

Fig. 110.—Slightly later stage than those of fig. 109; a little cytoplasm is now evident within the host cell. × 2000.

Fig. 111.—The portion of the nucleus which has entered is expanding into a structure of irregular form: both blepharoplasts and the strands connecting them to the nucleus are visible. × 2000.

Fig. 112.—The nucleus of the zygote lies unusually close to the surface of contact of zygote and host plant; no nuclear projection can be seen. × 2000.

Fig. 113.—Two later stages in the entry of nucleus and cytoplasm. × 2000.

Figs. 114*a*, *b*.—The zygote nucleus as it emerges from the pore is very irregular in shape. × 2000.

Fig. 115.—The nucleus is becoming spherical, but a portion is still extended towards the point of entry. × 2000.

Figs. 116-118.—Three late stages in the entry of the cytoplasm of the zygote after the nucleus has entered. × 2000.

Fig. 119.—The last portion of the cytoplasm to enter appears as a small granule outside the cell; plasmolysis has occurred. × 2000.

General Appearance of the Resting Sporangium in the Tumour.

Fig. 120.—Epidermal region of tumour bearing resting sporangia in a fairly advanced state of development; the zoospore-primordia are large; the original nucleus of the sporangium is shrunken and the epispore has been formed. × 170.

Fig. 121.—A very young resting sporangium which has passed down the host cell as far as the nucleus. × 400.

Fig. 122.—The young sporangium is now on the lower side of the host nucleus; a few chromatin granules have been given off into the cavity of the nucleus of the sporangium; host cell division has not yet taken place. × 400.

Fig. 123.—Host cell division has taken place and the sporangium lies in a sub-epidermal cell; chromatin granules have been formed in the cytoplasm of the sporangium, and a small amount of linin may be seen in the cavity of the nucleus. × 400.

Fig. 124.—The formation of the true membrane of the sporangium has begun; the nucleus has enlarged and contains more linin. × 400.

Fig. 125.—A second division of the host cell has occurred and the sporangium now lies in the third layer of cells; the contents of the host cell are reduced to a thin layer of protoplasm covering the sporangium and the host walls; the granules in the cytoplasm of the sporangium are conspicuously large; the membrane has almost reached its final thickness. × 400.

Fig. 126.—The dead contents of the host cell have been deposited as an epispore upon the sporangium; the wide clear central region of the epispore is formed from the sap region of the host cell; the more deeply staining inner and outer surfaces of the epispore arise from the host protoplasm. The outer layer of the true sporangial membrane is thick and is shown lightly shaded in the figure; the inner layer is too thin to be visible under the present magnification; the nucleus is shrunken and irregular in form, the nucleolus is colourless, and the nuclear cavity is filled with linin; the zoospore-primordia have enlarged greatly. × 400.

The Development of the Resting Sporangium.

Fig. 127.—A very young stage; the nucleus is a spherical chromatin globule lying free in the cytoplasm. × 1650.

Fig. 128.—A clear area has appeared round the chromatin globule. × 1650.

Fig. 129.—The clear area round the chromatin globule has widened, and linin threads pass across it; a definite nuclear membrane may be assumed to exist from this stage onwards. × 1650.

Fig. 130.—A stage immediately before the first nucleolar discharge begins; the nucleolus stains deeply at its periphery. × 1650.

Fig. 131.—An early stage of the first nucleolar discharge; small granules have been formed on a limited area of the nucleolus. × 1650.

Fig. 132.—The first nucleolar discharge; the granules have increased in size and now form a compact mass. × 1650.

Fig. 133.—The first nucleolar discharge; the chromatin of the projection is being dissolved and a linin-like substance is revealed between the isolated granules. × 1650.

Fig. 134.—A slightly later stage than fig. 133; the nucleolus stains deeply but it has been reduced to an oval shape during the discharge. × 1650.

Fig. 135.—First nucleolar discharge; the chromatin has completely disappeared from the nuclear cavity and only linin remains. × 1650.

Fig. 135*a*.—The second nucleolar discharge. Stages similar to those of figs. 131–135. × 1650.

Fig. 136.—The formation of the chromatic granules in the cytoplasm of the sporangium. *a*. Four large condensation areas are shown; minute granules may be detected on their strands. × 2000. *b*. New, smaller areas have appeared between the original ones. × 2000.

Fig. 137.—The third nucleolar discharge beginning; the granules have developed on the nucleolus; chromatic granules are now present in the cytoplasm of the sporangium. × 800.

Fig. 138.—The sporangial membrane although thin is now distinct; the nucleolus is still undergoing the third discharge and the chromatin of the projection is becoming dissolved; the nucleus has now reached its maximum size. × 800.

Fig. 139.—The third nucleolar discharge is completed; the chromatin has dissolved and only linin remains; the granules in the cytoplasm are larger and more numerous than in previous figures, and the membrane is denser. × 800.

Fig. 140.—Slightly later stage than that of fig. 139; the granules in the cytoplasm are more numerous, and the membrane is thicker. × 800.

Figs. 141–143.—The early, mid, and late phases of probably the fourth nucleolar discharge; the granules in the cytoplasm are increasing in size and in number, and the membrane of the sporangia is becoming thicker; in fig. 143 the beginning of the deposition of the host cytoplasm upon the sporangium can be seen; × 800. Fig. 143*a* shows the differentiation of structure now visible in the granules of the cytoplasm. × 1650.

Fig. 144.—The granules of the cytoplasm at a later stage than in fig. 143*a*; all now show differentiation of structure; some of the larger are multiplying by constriction. × 1650.

Fig. 145.—The chromatin of the zoospore-primordia (*i.e.*, of the enlarged granules in the cytoplasm) has greatly increased in amount. × 1650.

Fig. 146.—Accumulation of chromatin at certain points of the zoospore-primordia causing them to become irregular. × 1650.

Fig. 147.—The giving off of the substance of the chromatin projections from the zoospore-primordia. × 1650.

Fig. 147*a*.—Many granules are being given off, and those already emitted lie between the primordia. × 1650.

Fig. 147*b*.—The granules which were given off have ceased to stain deeply and have almost disappeared; occasional granules are still being emitted; the chromatin of the primordia is much reduced in quantity. × 1650.

Fig. 148.—A few colourless irregular structures on the strands of cytoplasm are all that remain of the emitted granules. × 1650.

Fig. 149.—The few small chromatin granules of the primordia are being replaced by single larger ones. × 1650.

Plate 16.

Fig. 150.—Stages in the constriction of the large primordia of which there are usually several in each sporangium.

Fig. 150*a*.—The difference in size between a normal and a large zoospore-primordium is shown. × 1650.

Fig. 151.—A zoospore beginning to expand as the result of absorption of water; the single chromatin granule has enlarged. × 1650.

Fig. 152.—The single chromatin granule forming the nucleus has given rise to several granules and a clear area has developed in their midst; the cytoplasm of the zoospore is much vacuolated. × 1650.

Fig. 153.—Mature zoospore with blepharoplast and the deeply-staining strand which connects it with the nucleus; the cytoplasm is now arranged in delicate strands round the periphery of the zoospore while the centre is occupied by a large vacuole. × 1650.

The Appearance of the Original Nucleus of the Resting Sporangium during the Later Stages of Development of the Zoospores.

Fig. 154.—The distribution of the linin matrix resulting from a nucleolar discharge, probably following the stage shown in the sporangium of fig. 143 ; wide spaces are present in the reticulum ; the nucleolus is vacuolated but still budding. × 800.

Fig. 155.—The distribution of linin has continued and the width of the mesh has increased. × 800.

The Formation of the Outermost Layer of the Wall of the Resting Sporangium.

Fig. 156.—A portion of the sporangium and host cell before the formation of the epispore (outermost layer) ; disintegration products of an oily nature have been formed in the protoplasm on the sporangium and in that on the host walls ; unbleached material. × 1650.

Fig. 157.—The vacuole region of the host cell has a milk white gelatinous appearance as its contents change to form the membrane ; the disintegration globules are conspicuous on the sporangium ; unbleached material. × 1650.

Fig. 158.—The membrane after it has become yellow in colour ; the disintegration globules will remain at the inner and outer surfaces of the newly formed layer ; unbleached material. × 1650.

The Division of the Infected Host Cell.

Fig. 159.—A small portion of the epidermal region of the host tissue when the wide-spread first division is taking place ; two walls have been formed parallel to the surface, and one is oblique ; the parasites lie in the lower ends of the host cells. × 400.

Fig. 160.—Four adjacent epidermal cells, each containing several parasites ; division of the host cells has not yet occurred ; all the parasites are smaller than the host nuclei. × 780.

Figs. 161–163.—Prophase, metaphase and telophase respectively of the division of the epidermal host cell ; in every case the parasites will be cut off in the inner daughter cell. × 780.

Fig. 164.—Division of a sub-epidermal cell ; the second wall will be set obliquely to the first division wall, and the parasite will then lie in the innermost cell. × 780.

Fig. 165.—Division (prophase) of a deeply placed host cell ; the sporangium has already formed a thick membrane. × 400.

Figs. 166, *a*, *b*.—Six parasites contained in adjacent sections of two daughter cells ; the parasites have been separated into two groups on the division of the original cell, and would have been further distributed by the repeated division of both daughter host cells. × 780.

Discharge of the Zoospores of the Resting Sporangia.

Fig. 167.—The innermost layer of the membrane of the sporangium has become strikingly hyaline ; the sporangium is distended and the zoospores, which are not shown in this figure, are separating from one another. × 780.

Fig. 168.—The conical projection, derived from the hyaline innermost layer of the membrane, the formation of which causes the sporangium to burst. × 780.

Fig. 169.—Ruptured empty sporangium ; the hyaline layer is swollen and lies in folds within the two outer layers of the membrane. × 780.

Fig. 170.—Surface view of a living sporangium during the maturation of the zoospore-primordia. × 780.

Structure and Entry of the Zoospore.

Fig. 171.—View of living zoospore ; the anterior refractive spot marks the position of the nucleus. × 780.

Fig. 172.—Zoospore, fixed and stained, showing nucleus and blepharoplast, and the deeply staining strand connecting the two. × 780.

Fig. 173.—Zoospores in a cleft on the surface of the host plant ; cilium-reduction can be seen in several cases. × 800.

Fig. 174.—Zoospore at rest on the host surface before entry begins ; nucleus, blepharoplast, and connecting strand are all visible. × 2000.

Figs. 175–177.—Stages in the elongation of the nuclear projection towards the surface of contact. × 2000.

Figs. 178, *a–d*.—Stages in the formation of the nuclear projection when the nucleus lies close to the surface of contact and elongation is thus relatively slight. × 2000.

Fig. 179.—The tip of the nuclear projection is pressed against the surface of the zoospore immediately before entry begins. × 2000.

Fig. 180.—The contraction of the cilium ; the nuclear projection reaches half way to the surface. × 2000.

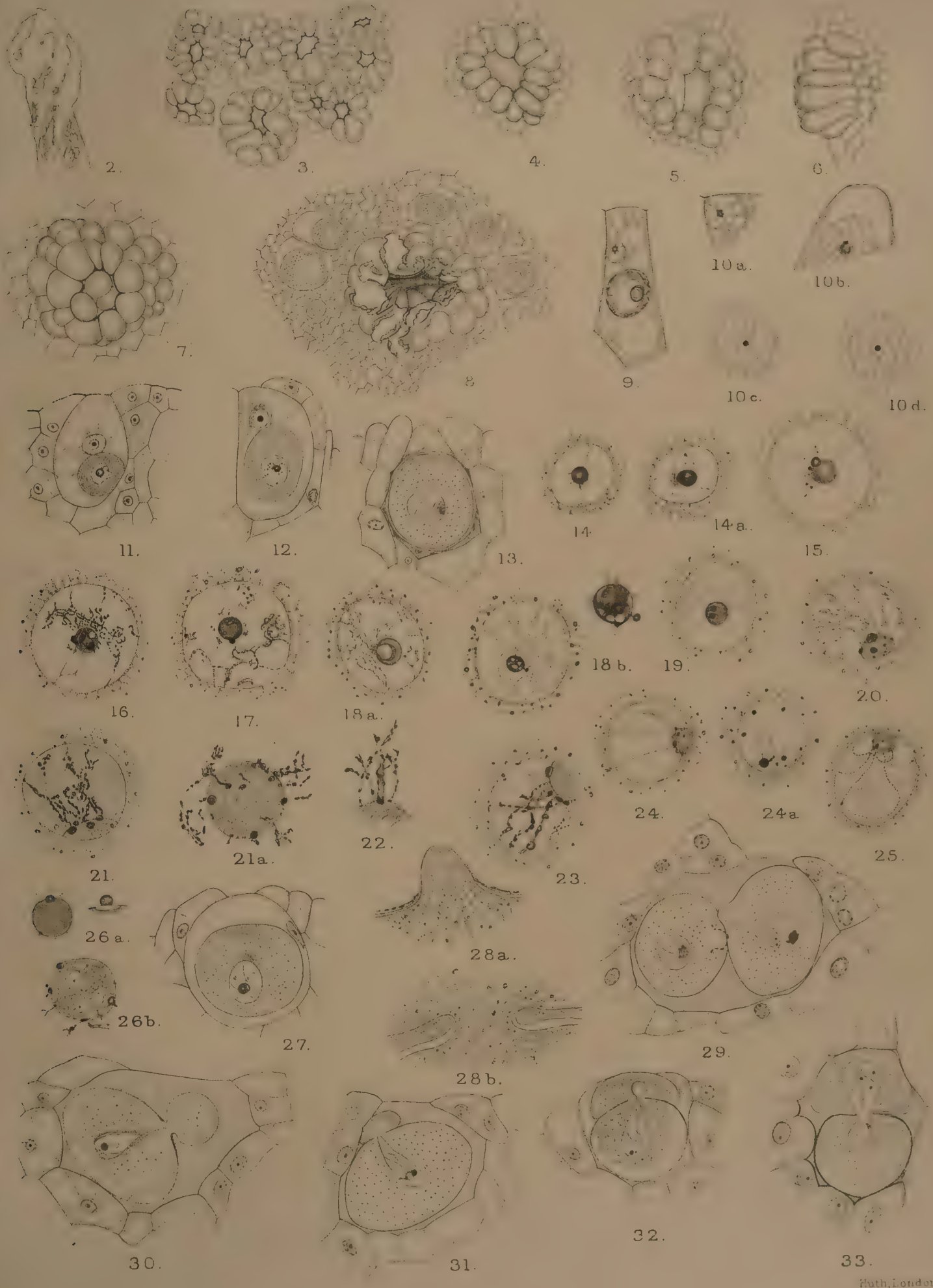
Fig. 181.—Entry into the host cell of the apical region of the nuclear projection. × 2000.

Fig. 182.—A small quantity of cytoplasm has passed into the host cell ; plasmolysis has occurred. × 2000.

Fig. 183.—In both examples the nucleus has almost completed its entry and only a small amount of cytoplasm remains outside. × 2000.

Fig. 184.—Entry completed ; the nucleus still extends towards the point of entry. × 2000.

Fig. 185.—A later stage than that shown in fig. 184 ; the nuclear extensions have now been withdrawn. × 2000.



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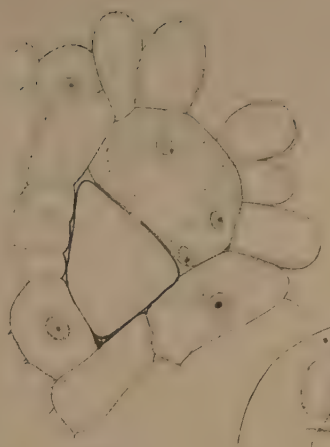
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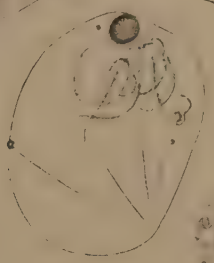
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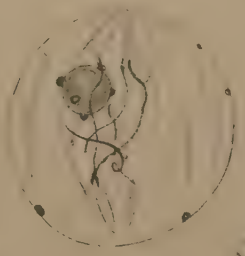
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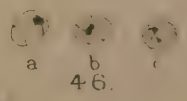
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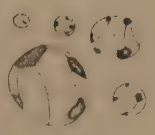
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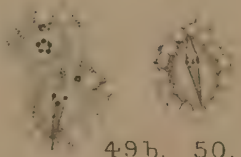
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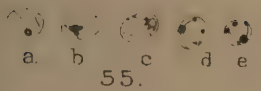
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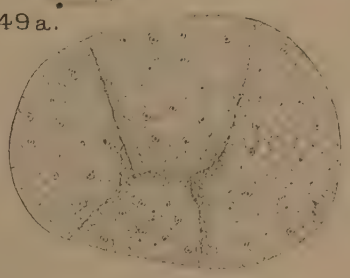
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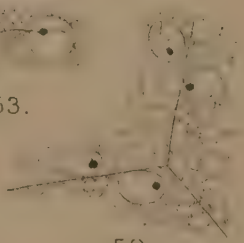
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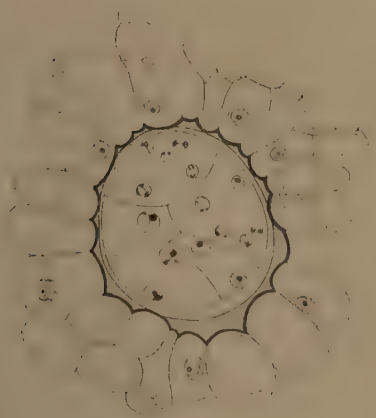
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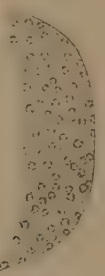
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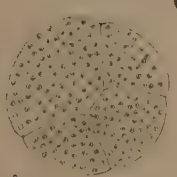
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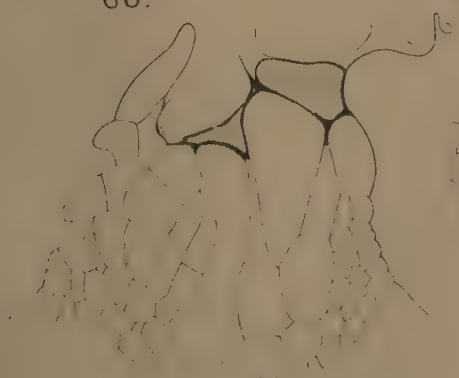
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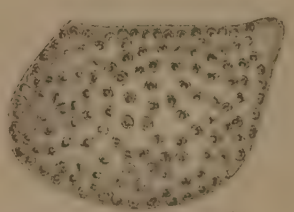
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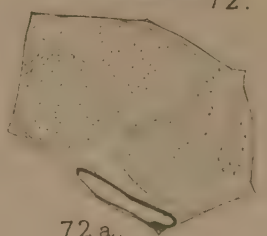
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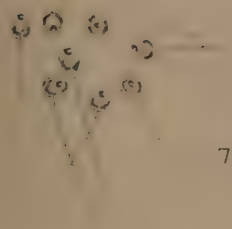

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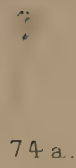

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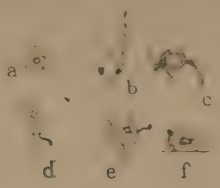

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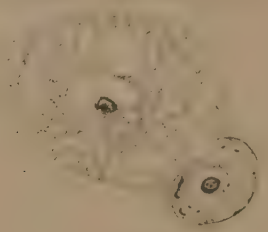

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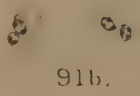

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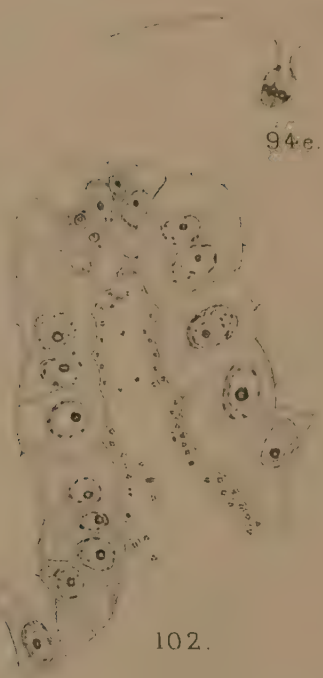

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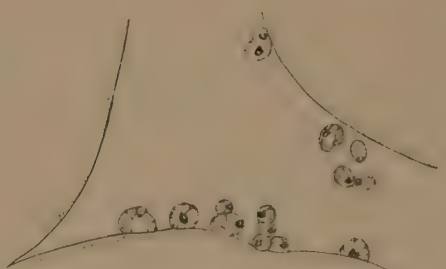

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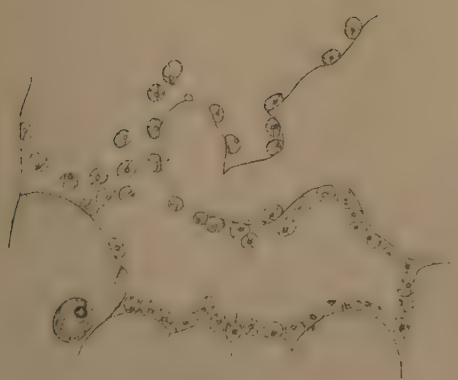

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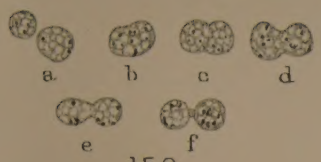

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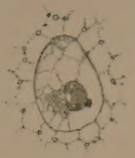

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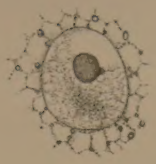

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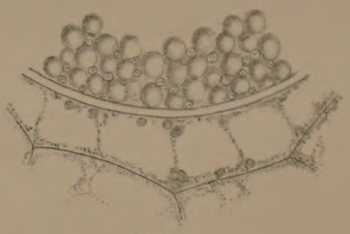

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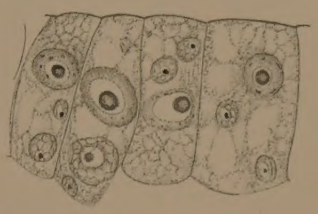

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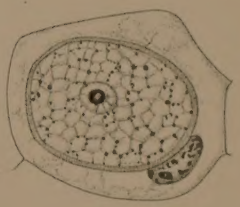

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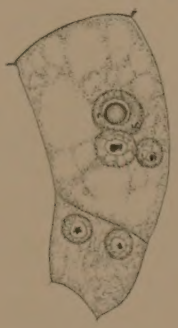

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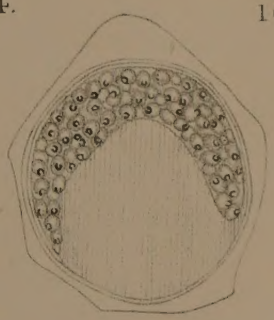

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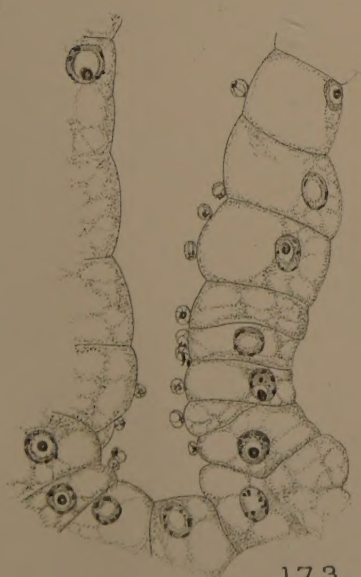

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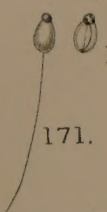

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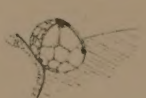

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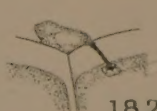

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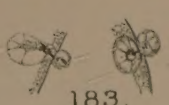

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